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Determinants and Developmental Consequences of Organic Contaminant Uptake in Nestling Insectivorous Birds

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**DETERMINANTS AND DEVELOPMENTAL CONSEQUENCES OF ORGANIC
CONTAMINANT UPTAKE IN NESTLING INSECTIVOROUS BIRDS**

by

Mary Sebastian M.

A Dissertation

Submitted to the Faculty of Graduate Studies
through the Department of Biological Sciences
in Partial Fulfillment of the Requirements for
the Degree of Doctor of Philosophy at the
University of Windsor

Windsor, Ontario, Canada

2010

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Declaration of Co-Authorship / Previous Publication

I. Co-Authorship Declaration

I hereby declare that this thesis incorporates material that is result of joint research, as follows:

The basic concept for this research is from a collaborative project between Dr. Judit Smits and Dr. Gary Bortolotti of University of Saskatchewan and Dr. Jan Ciborowski and I, Mary Sebastian from University of Windsor. The results of that study have been published (Smits *et al.* 2005; Appendix 1). I helped in data collection especially the diet determination part and analyzed data and helped in preparing the manuscript.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis, and have obtained written permission from each of the co-author(s) to include the above material(s) in my appendix of my thesis.

I certify that, with the above qualification, this thesis, and the research to which it refers, is the product of my own work.

II. Declaration of Previous Publication

This thesis includes 1 original paper that has been previously published in peer reviewed journals, as follows:

Thesis	Publication title/full citation	Publication status*
Appendix 1	Smits, J.E., Bortolotti, G.R., Sebastian, M., Ciborowski, J.J.H. 2005. Spatial, temporal, and dietary determinants of organic contaminants in nestling tree swallows in Point Pelee National Park, Ontario, Canada. <i>Environmental Toxicology and Chemistry</i> 24: 3159-3165.	Published in Environmental Toxicology and Chemistry

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ABSTRACT

This research assesses the ecotoxicology of three avian species in their natural environment. Field studies investigated potential toxicological effects of organochlorine pollutants including polychlorinated biphenyls (PCBs) and dichlorodiphenyl trichloroethane (DDT) and their metabolites on wildlife with passerine nestlings as model organisms. For organisms at higher trophic levels, especially terrestrial ones the major route of exposure to persistent pollutants is food. Consequently, I estimated the diet of nestlings of populations of three species - tree swallows (*Tachycineta bicolor*), purple martins (*Progne subis*) and house wrens (*Troglodytes aedon*) at Point Pelee National Park, Ontario, Canada and their contaminant contents. Another route of exposure in oviparous organisms is through their eggs, so the contaminant contents in the eggs of each population were analyzed. I measured nestlings' growth rates in the field and estimated consumption rates from those values. The contamination loads were estimated by multiplying consumption rates by food-specific contaminant concentrations and combined with egg-acquired burdens in a bioenergetics-based model to predict the bioaccumulation levels of various PCB congeners and DDE in each species. These estimates were compared with tissue contaminant concentrations of nestlings sacrificed at their fledging age. The deviations between the predicted and observed values reflected the biotransformation and absorption abilities of the nestlings.

To measure nestlings' biological responses to organochlorine pollutants, house wren nestlings' diet was supplemented with *Hexagenia* mayflies collected from three locations containing different PCB burdens. Nestlings fed mayflies collected from a heavily contaminated site had significantly reduced relative growth rates and enlarged livers and hearts relative to controls (whose diets were not supplemented with mayflies) and to individuals fed mayflies from less contaminated sites. However, nestlings fed 'reference' mayflies that had been spiked with a PCB mixture grew at the same rate as nestlings fed with mayflies collected from a moderately polluted location, suggesting that contaminants other than PCBs were responsible for the observed impairments.

The results of this thesis demonstrate that the accumulation of pollutants and its effects on nestling passerines in natural habitats are based on composition of food,

contributions from maternal burden accumulated from breeding and overwintering locations and bioenergetics of nestlings including biotransformation capacities.

This thesis is dedicated to the nestlings and parents of the nestlings used in this study.....

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This research was funded through grants from the Natural Sciences and Engineering Research Council of Canada and from Syncrude Canada Ltd. to J.J.H. Ciborowski, and the Purple Martin Conservation Association to M. Sebastian. I am grateful for the financial support received from the University of Windsor in the form of various scholarships, travel awards, and an Ontario Graduate Scholarship for Science and Technology.

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Chapter 1: General introduction

The objectives of this thesis are to evaluate different determinants of the bioaccumulation of organochlorine pollutants (dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCB)) in passerine nestlings and to assess exposure effects of these pollutants by using biomarkers.

Organochlorine pollutants like DDT and PCB are commonly known as persistent organic pollutants (POPs). These chemicals are of great concern in ecosystems as they resist degradation and they are bioaccumulative and toxic (Tanabe, 2002; Kelly *et al.*, 2004; Beyer and Biziuk, 2009). They are found in every habitat and in every compartment on the planet (Fisk *et al.*, 2001) and in most biological systems (Safe, 1994). They can be seen in regions far from their source of origin because these chemicals can circulate globally through the atmosphere (long-range atmospheric transport, LRAT), oceans, and other pathways (US EPA, 2006; Beyer and Biziuk, 2009). Organochlorines can modulate immune systems in wildlife and humans (Grasman and Fox, 2001). High concentrations of organochlorines in animals can affect their endocrine, reproductive (Dawson, 2000) and nervous systems (Walker, 2002).

POPs in animals

Since they are highly hydrophobic, POPs bioaccumulate in organisms' fatty tissues (Drouillard *et al.*, 2004); these compounds are great threats to organisms at higher trophic levels, such as predatory fish, birds, and mammals including humans (Kelly *et al.*, 2004). Bioaccumulation of a substance is a compound's capacity to accumulate in the tissues of organisms, either through the organism's direct exposure to water, air or soil, or through consumption of food, i.e., uptake from the environment by all possible pathways (Jørgensen and Halling-Sørensen, 1998; Mackay and Fraser, 2000). Bioaccumulation is calculated as a ratio termed the Bioaccumulation Factor (BAF). BAF in a steady-state situation is expressed as the ratio of concentration in the organism to concentration in the medium and the food to which the organism is exposed. So it represents bioconcentration plus biomagnification.

Bioconcentration is the net accumulation in and on an organism from its ambient environment, i.e., from the respiratory medium through respiratory surfaces (Gobas *et al.*, 1999; Mackay and Fraser, 2000; deBruyn *et al.*, 2006). Biomagnification is defined as the process by which a chemical in a consumer organism achieves a thermodynamic activity (often measured by the lipid normalized concentration or fugacity) in excess of that in its diet or an increase in contaminant concentration from one trophic level to another due to accumulation from food (Newman, 1998; Gobas *et al.*, 1999; deBruyn *et al.*, 2006). Fugacity is expressed as the “escaping tendency” of the chemical from its medium or in essence it is a chemical’s concentration normalized to its solubility in the medium in which it resides (deBruyn *et al.*, 2006).

Bioconcentration depends mainly on the octanol:water/air partitioning coefficient (K_{ow} and K_{oa}) for hydrophobic chemicals (Mackay and Di Guardo, 1995). Chemicals with high K_{ow} have only a small fraction dissolved in water and thus are not readily taken up by organisms in this phase. The degree of bioconcentration at steady state can be represented by the bioconcentration factor (BCF), which depends on the rates of absorption and elimination. $BCF = k_1/k_2 = C_a/C_w$ where k_1 is the uptake rate and k_2 is the clearance rate and C_a and C_w are the concentrations of the chemical in the organism and water, respectively (Jørgensen and Halling-Sørensen 1998; Mackay and Fraser, 2000).

In terrestrial habitats, especially in higher trophic level organisms such as birds, biomagnification is the major route for bioaccumulation of contaminants. Many important processes such as dietary absorption/assimilation, different elimination pathways, metabolic transformation and growth dilution are important determinants of biomagnification in these organisms (Kelly *et al.*, 2004). An organism’s xenobiotic metabolizing system is a major determinant of the bioaccumulation of pollutants. Biomagnification can be quantified by measuring the biomagnification factor (BMF). The BMF is the lipid-corrected chemical concentration in an organism divided by the lipid-corrected chemical concentration in its food, i.e. $C_{predator}/C_{prey}$ or $C_{organism}/C_{food}$. Animals that are at higher trophic positions have proportionately more lipid per unit body mass than organisms at lower trophic levels; this accounts for higher contaminant concentration in organisms higher in a food web (Kucklick *et al.*, 1996; Mackay and

Fraser, 2000). Biomagnification occurs when the fugacity of hydrophobic chemical increases in a predator's lipids when prey containing fat-soluble contaminants are digested and absorbed in the gastrointestinal tract. Fugacity of a chemical in a given medium depends on its molar concentration and the fugacity capacity of the medium in which the chemical is dissolved (Kelly *et al.*, 2004). Russell *et al.* (1999) found that chemicals with high hydrophobicity and thus high- K_{ow} ($\log K_{ow} > 6.3$) were biomagnified in food webs they studied; they observed no biomagnifications for low- K_{ow} chemicals ($\log K_{ow} < 5.5$) in the food web, and chemicals with $\log K_{ow}$ between 5.5 and 6.3 showed very low level of biomagnification.

Hydrophobicity and K_{ow} increase if the toxic substance has an aromatic ring and when the hydrogen atoms in the ring are substituted by chlorine atoms. For example, if hydrogen in a benzene ring is replaced by chlorine atoms, each chlorine atom increases $K_{ow} \approx 5$ fold (Mackay and Di Guardo, 1995). PCB congeners, which have biphenyl rings in their structure, show higher biomagnifications with more chlorination. The substitution position in the biphenyl ring also affects hydrophobicity. If there are open *meta-para* positions in the biphenyl ring of PCBs then they will be easily eliminated from animal tissues and will show less biomagnification. Depending on their elimination/retention they show different levels of biomagnification. Studies conducted by Drouillard *et al.*, (2001; 2007) and Clark *et al.*, (1987) showed that PCB congeners and other organochlorines can be grouped into three categories based on the number of open *meta-para* positions on their biphenyl ring and their half-life in different organisms. These groups are rapidly cleared chemicals, intermediate cleared and slowly cleared.

An assessment of the level and toxicity of POP chemicals requires a clear understanding of the mechanisms in food chain energy transfer. Determination of an organism's food items and contaminant level in the food are essential to determining biomagnification in higher trophic level organisms (Borga *et al.*, 2001). Consumption rate determines the quantity of contaminants that enter the body, and the assimilation rate determines the amount actually assimilated by the organism. The consumption rate is determined by the energy requirements of the organism. So bioenergetics of organisms provides an important context in biomagnification studies. It is important to study the contribution from maternal tissues to offspring, too, because it is a significant

determinant in bioaccumulation. Another important area of investigation is to measure the biological response of wildlife to their burden of persistent organochlorine pollutants or the expression of biomarkers that could be used to assess toxic effects of environmental contaminants in wildlife.

Focus of the project

My project is focused on four major areas:

1. the role of maternal contribution (egg in oviparous animals) to contaminant burdens of young organisms;
2. the role of insects (as diet items) in the transfer of environmental contaminants to their consumers;
3. the mechanisms regulating the bioaccumulation of organochlorines to animals, especially the role of bioenergetics of the species in contaminant bioaccumulation and
4. the measurement of birds' biological responses to contaminants by monitoring biological end points (biomarkers).

My study organisms are insectivorous passerines preying upon both aquatic and terrestrial insects. The studies were conducted in Point Pelee National Park of southern Ontario, Canada.

Point Pelee National Park

Point Pelee National Park, the southernmost tip of Canadian mainland is a paradise of migratory birds. This coastal peninsula is internationally famous for spring and fall migration of birds and autumn monarch butterfly migration (Proctor and Lynch, 1993; Stewart, 1977; Hebert, 2002; Parks Canada, 2003). Point Pelee occupies an area where the Atlantic and Mississippi flyways overlap. These two flyways are important continental migratory routes for many bird species (Fletcher, 1997). As a land mass that extends over 10 km into Lake Erie, many birds use it as a stopover for rest after their long flight across the lake. About 90 species, including many passerines remain and breed in

the park. Tree swallows, purple martins and house wrens are among the species that breed in the park.

Biological importance of the park

Point Pelee is the second smallest national park in Canada, with an area of only 15 km². The landscape is a mix of marshes, forests, fields, and beaches within the Carolinian zone (Stewart, 1977; Hebert, 2002; Parks Canada, 2003). This provides a unique habitat in Canada, suitable for many types of fauna and flora. The biodiversity in this park is rich compared to many other larger national parks in Canada (Stewart, 1977; Parks Canada, 2003). Point Pelee is an area of high biological activity. Between 6,000 and 10,000 of an estimated 60,000 species of insects in Canada have been identified in Point Pelee National Park (S. Marshall, University of Guelph, unpubl., cited in Tong, 2000). All three avian study species are insectivorous, making this park an appropriate area to study insects and their predators.

Contaminants in the park area

Farming was once a major activity in Point Pelee. Many parts of the park area were cleared and drained for farming, and evidence of abandoned orchards can still be seen. There was heavy use of DDT (dichlorodiphenyltrichloroethane) in the park by farmers during 1950s and 1960s to control agricultural pests and biting insects. In the 1970s, DDT use was banned from the park, but the levels of DDT in the park environment remain well above government guidelines. Tissue analyses of some park species have shown high levels of DDT (Natural Heritage, Soil Contamination Study, 2001). Analysis of soil samples of the park by the same study has revealed four areas where DDT levels are above recommended government agency guidelines. The presence of large quantities of residual DDT in the soil of Point Pelee reflects the extremely high past application rates (Harris *et al.*, 2000). DDT is highly persistent so it still has strong bioaccumulation capacity (Connell *et al.*, 2002).

Lake Erie sediments have levels of PCBs that vary among locations, reflecting different sources (Gewurtz and Diamond, 2003; Marvin *et al.*, 2004; Heidtke *et al.*, 2006). The immature stages of most aquatic insect species spend most of their larval life dwelling and feeding in the sediments. Depending upon the location of the sediments in which they develop, they will have varying loads of persistent contaminants in their tissues (Gobas *et al.*, 1989; Corkum *et al.*, 1997).

DDT (dichlorodiphenyltrichloroethane)

DDT is an organochlorine compound produced as a mixture of *p,p* isomer and *o,p'* isomer. The *p,p* isomer is the largest component. The major environmental breakdown products of DDT are dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD). DDT was used as an insecticide mainly to control mosquito-borne malaria. It was also used against biting insects and as a pesticide in the field. DDT was completely banned from Canada in 1985 (Pesticide News, 1998). K_{ow} values of DDT, DDE and DDD are 6.38, 6.57 and 5.9 (Hoke *et al.*, 1997). When it is expressed as Σ DDT in this thesis, it will represent the sum of components of DDT and their metabolites.

PCB (polychlorinated biphenyls)

PCBs are a mixture of 209 distinct congeners consisting of a biphenyl backbone to which 1 to 10 chlorine atoms are bound at different positions. PCBs have the chemical formula $C_{12}H_{10-x}Cl_x$, where $x = 1-10$. In Canada, the manufacture, import and sale of PCB was prohibited in 1977, and its release into the environment was banned in 1985 (Environment Canada, 2006). PCBs were used for many different industrial purposes. The commercial success of PCB was based on its properties of nonpolarity, lipophilicity, electronegativity, stability, and heat resistance. However, these qualities make them very persistent, bioaccumulative and harmful (Hooper *et al.*, 1990). The use of the term “ Σ PCB concentration” in this thesis represents the sum of 38 congeners analyzed and quantified in different kinds of samples used for this study.

PCB congeners and other organochlorines analysed for this study were categorized based on expected persistence patterns in birds and based on known elimination rates and time to steady state measured for other species as follows:

Rapidly cleared with half-lives 10-60 d: OCS, dieldrin, PCB 18, 17, 31/28, 52, 49, 44, 70/76, 87, 95, 101, 110, 149, 151, 174

Intermediate cleared with half-lives >60-150 d: HCB, PCB 74

Slowly cleared with half-lives > 150 d: oxychlordane, mirex, DDE, PCB 85, 99, 105, 118, 128, 130, 137, 138, 146, 153/132, 156, 158, 171, 177, 178, 180, 183, 187/182, 195, 194, 200, 201, 203, 206, 209 (Clark *et al.*, 1987; Drouillard *et al.*, 2001; 2007).

Passerines

A passerine is a bird of the order Passeriformes. They are also known as perching birds or song birds. They are one of the most successful vertebrate orders with approximately 5,899 known species, in 1,207 genera and 82 families (Perrins, 2003). All passerine nestlings are altricial. Altricial nestlings are nidicolous and need extensive parental care at the early stages of development. They are totally dependent on their parents for food and other needs including temperature maintenance as they are born naked, blind, and completely helpless; however, the chicks grow very rapidly and fledge within a very short time period.

Study species

Tree swallows (*Tachycineta bicolor*) and purple martins (*Progne subis*) belong to the family Hirundinidae and the house wren (*Troglodytes aedon*) belongs to Troglodytidae under the suborder Passerida in the order Passeriformes. The family Hirundinidae is characterized by their adaptation to aerial feeding, and species are known as swallows and martins. Within the Hirundinidae, the name swallows tends to be used for the more fork-tailed species and the name martin for the squarer-tailed species.

Tree Swallows are widely distributed passerine birds and a very hardy species. Their name *bicolor* is derived from their body colors of steely greenish

blue above and white below. Adults typically weigh 18 to 25 g. Clutch size varies from 4 to 8 eggs (Dunn and Hannon, 1992; Dunn *et al.*, 2000) and weigh approximately 1.4 to 1.9 g. Nestling hatching occurs 11 to 19 days after laying, with a hatching weight of 1.5 to 1.7 g and fledging occurs from 15 to 25 days (Robertson *et al.*, 1992). Tree swallow parents feed their nestlings and themselves extensively on emergent aquatic insects. A previous study conducted by Smits *et al.*, (2000) showed that the food samples collected from 50 different sites for tree swallows were dominated by aquatic insects and which accounted for approximately 84% of their diet. Dipterans (mainly Chironomidae) constituted 90% of the diet in many locations in a study by Nicholas *et al.*, (1995). They accept nest boxes (Roof and Harris, 2000 and Bishop, *et al.*, 1995; 1999). Adult swallows forage within limited areas when providing for their young, making them model organisms to study local contamination.

Features of model organisms include:

1. short generation time
2. small adult size
3. ready availability
4. tractability

Tree swallows accept and breed in nest boxes, which makes them an easily available and traceable study species for avian biologists (Jones, 2003). Purple martins and house wrens also share the above qualities of tree swallows and thus they are also suitable for biological studies.

Purple martins are the largest of Hirundinidae and are distributed throughout North America. Purple martins tend to capture insects by aerial hawking over terrestrial rather than wetland habitats, foraging on swarms of terrestrial insects (Sibley, 2001). Purple martins depend on birdhouses and gourds provided by humans for nesting. Adult males are dark-bellied with glossy blue-black and females are characterized by distinct brownish or grayish collars around their nape (Brown, 1997). Adult purple martins weigh around 45 to 55 g. Females lay 4 to 5 eggs and weigh from 3.5 to 4.5 g. The

incubation period is around 15 to 20 days and fledging occurs from 27 to 36 days. New hatchlings weigh about 2.8 g (Brown, 1997).

Adult house wrens usually weigh 10 to 12 g. House wrens have long, curved bills and perch with their tail held erect. This is a characteristic feature of this species. Their body is uniformly brown with very fine darker brown stripes. Their throats and chests are light grey, and may have some black or dark brown spots on their flanks, tails, and wings. Above their eyes there may be a distinct white eyebrow like stripe (Johnson, 1998). These birds are also distributed throughout North America. Their clutch size varies from 4 to 8, and the eggs weigh from 1.45 to 1.9 g. The eggs hatch from 9 to 16 days and fledge from 16 to 18 days. The hatchlings weigh around 1.2 g (Johnson, 1998). These birds construct their cup nest in different types of cavities - either natural or man-made, and will readily use nest boxes if they are provided. House wrens are insectivorous but they are gleaners, i.e., they capture insects from the bushes.

The above three species have different birth weights, adult weights, different incubation and fledging periods. Even though they breed in the same area and are grouped under same heading (insectivorous passerines) they have their own characteristics which make them distinct from one another. Global population trends for tree swallows, purple martins and house wrens have not been quantified, but these species are not included in the list of species that are endangered and enlisted as groups of least concern (Birdlife International 2004 a, b, c).

Background information for this thesis

This study started as a collaborative project between Dr. Judit Smits and Dr. Gary Bortolotti of University of Saskatchewan and Dr. Jan Ciborowski and myself from the University of Windsor. Dr. Smits and her team studied tree swallow nestlings at DDT-contaminated and uncontaminated areas in Point Pelee National Park for evidence of health impairment, measured using physiological and immunological endpoints. Contemporaneously, we studied the trophic dynamic aspects; i.e., the bioaccumulation of pollutants that could be manifested as physiological and immunological damage. The results of that study have been published (Smits *et al.*, 2005 Appendix 1; Papp *et al.*, 2007). The major findings of that study, (listed below), led me to pursue further research:

1. Nestlings had detectable levels of PCBs and DDT and its metabolites
2. Σ DDT concentrations in nestling tissues were higher in contaminated sites than reference sites
3. Σ DDT concentrations correlated with terrestrial insect fractions in nestlings' diet
4. Σ PCB concentrations in the nestling tissues did not vary significantly among sites
5. Σ PCB concentrations correlated with relative biomass of aquatic insects (especially mayflies (*Hexagenia* spp. (Ephemeraidae)) in the diet
6. Contaminant burdens in tree swallow nestlings strongly reflected the amount and types of contaminant in their food

I confirmed the dietary composition and its association with biomagnification by looking at the composition of insects in nestlings' food boluses and by comparing the PCB and DDT concentrations in different groups of local insects and nestlings. An important discovery was the relative domination of contaminant burden by either DDT or PCBs depended more on the types of food that the parents provided to their young than on where the nests were located. Some nestlings at the DDT-contaminated sites had been fed predominantly on mayflies, and tended to have low DDT burdens. Nestlings at sites slightly away from Lake Erie that were given food rich in terrestrial insects had elevated DDT burdens. Even though tissue concentrations of PCBs were not high enough to be detrimental, if by chance mayflies emerging from Lake Erie (from polluted locations) are more contaminated than Point Pelee specimens, this could lead to an augmentation of contaminant burden in the nestlings and induce contaminant-related impairments in nestlings. I am interested in measuring contaminant burdens without harming the bird, *i.e.*, in a non-destructive tissue. So the consumption–biomagnification–biomarker triad is my focus.

The underlying goal of my research is to develop ways to identify or predict nestling exposure to bioaccumulative pollutants that have similar modes of action without causing harm to the birds. Such protocols would provide authorities with inexpensive tools to regularly track changes resulting from mitigative measures such as sediment

remediation. My research aims at evaluating non-destructive biomarkers of biological responses to the stress caused by exposure to compounds like PCBs and DDT. My goal in this study was to develop a diagnostic tool for PCB biomagnification in nestling body tissues by measuring a morphological biomarker. The experiment planned was an evaluation and validation of that tool.

Outline of the thesis

This thesis has two sections. The first part deals with the determinants in the accumulation of pollutants (Chapters 2-5). The second is an evaluation of the effects of exposure of pollutants (Chapters 5 and 6). The important study areas with the focus of different chapters are presented as a flowchart (Figure I).

For birds, the first and foremost source and determinant of contaminant accumulation by nestlings is the eggs from which they develop, i.e., the nutrients they receive from their mothers. In the second chapter, the egg contaminant patterns of the three study species were explored.

In birds, the next important determinant is their diet. In insectivorous birds the type of insects, the habitat from which the insects emerge (aquatic and terrestrial) and insects' trophic position etc., are important factors that determine the contaminant accumulation. In the third chapter of my thesis, the major diet determined for nestlings of all three passerines by different methods are described. For tree swallow nestlings, a study was conducted to infer the diet for the entire nestling period by using stable carbon and nitrogen isotope techniques (Appendix 2)

The fourth chapter is the most important section, which deals with the important predictors of contaminant accumulation - the bioenergetics. This chapter is also devoted to exploring the species' differences in accumulation patterns based on their biotransformation capacity, depending on the K_{ow} and persistence of the different pollutants, the extent of onsite accumulation relative to their accumulation from overwintering locations through eggs. Using bioenergetic parameters from literature and absolute growth observed in the field, I calculated the consumption rate for the nestlings of the three species. After determining the contaminant content in the diets, the eggs, and the amount food consumed, I predicted accumulation possibilities for DDE and for

different PCB congeners for nestlings of the three study species and compared them with the observed values for respective species.

In the fifth chapter I tried to experimentally induce different levels of bioaccumulation of PCB in nestlings and evaluated some nondestructive biomarkers of exposure. This was done by feeding wren nestlings in the field with mayflies (a natural food item of the nestlings) containing different PCB concentrations in their tissues. The daily ration was calculated using the consumption rate from the previous chapter.

The sixth chapter is a review of different avian nondestructive biomarkers or biological end points that are used by different avian ecotoxicologists.

The final chapter summarizes and synthesizes the findings of my work.

As these chapters are planned as manuscripts for journals I will repeat some methodological details from chapter to chapter.

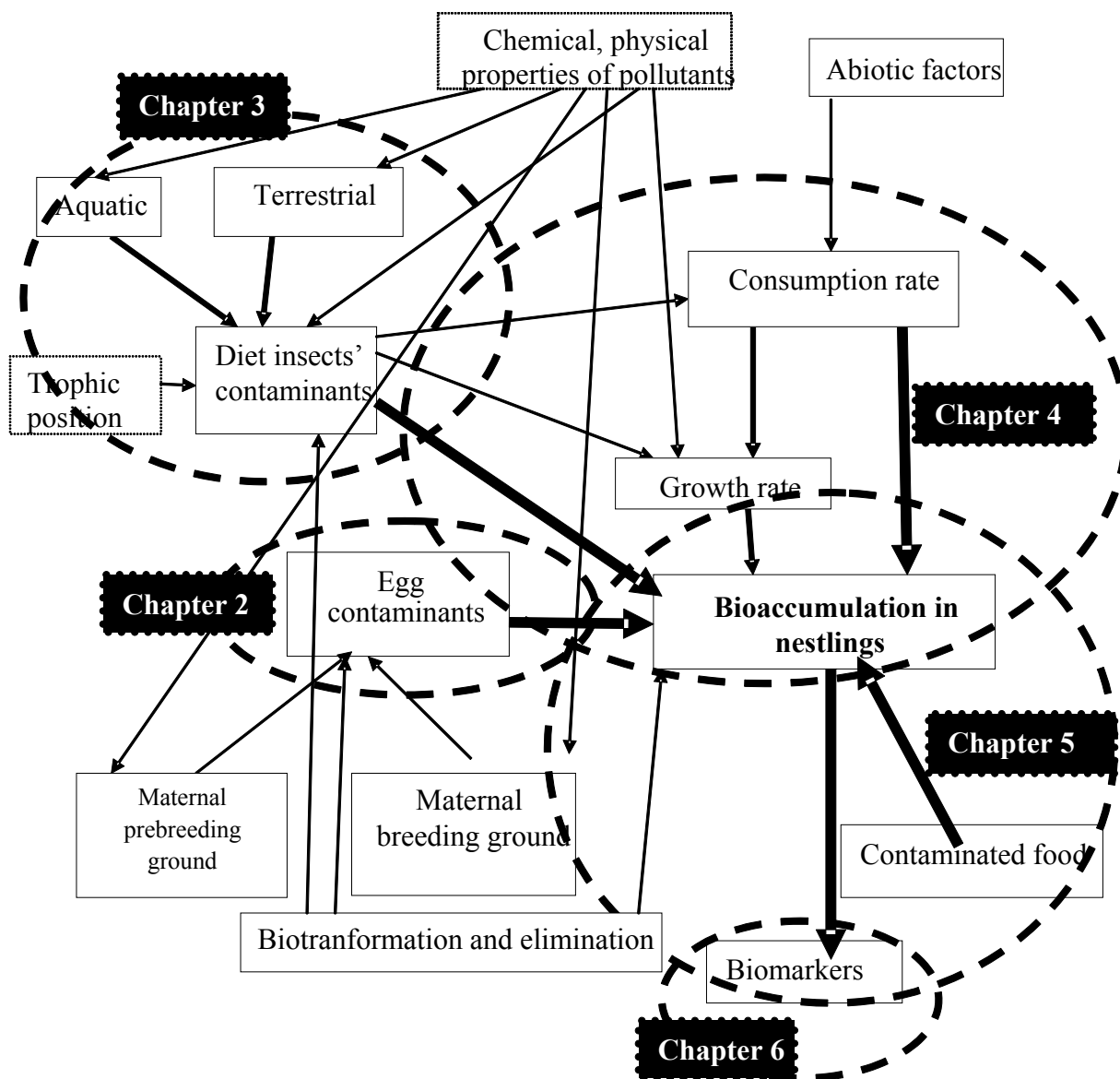


Figure 1. Flowchart of different chapters in the thesis with focus areas enclosed in dashed circles. The bold arrows represent contributing factors to the major focus "the bioaccumulation of organochlorine pollutants" studied in passerine nestlings

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Chapter 2: Polychlorinated biphenyl congener profile and organochlorine pesticides in the eggs of three passerine species breeding in Point Pelee National Park, Ontario, Canada

Introduction

The study of persistent and bioaccumulative organic chemicals in eggs is relevant to the study of avian toxicology because such chemicals are often subject to maternal transfer to eggs and because the embryo, prenatal and early postnatal periods of development are considered highly sensitive to toxicant exposures (Fossi, *et al.*, 1999; Drouillard *et al.*, 2003; Martinez-Lopez *et al.*, 2007; Spears *et al.*, 2008). Juvenile birds are also frequently used as biomonitors to gauge contaminant exposures at the breeding grounds. Juvenile birds receive organic-contaminant-mass from both maternal sources via egg yolk as well as site specific diet contributions from the breeding site. Thus, determination of bioaccumulation patterns of congener specific polychlorinated biphenyl (PCB) and other organochlorine (OC) contaminants and their metabolites in avian eggs, especially those of migratory birds, are needed to understand the relative importance of sources of chemical uptake in post-hatched and fledgling juvenile birds.

The contaminant concentration and chemical signatures in eggs tend to reflect those of the adult maternal tissues at the time of egg formation (Braune and Norstrom 1989; Drouillard and Norstrom, 2001; Alcock *et al.*, 2002; Verboven *et al.*, 2009). Bioaccumulation of organochlorine compounds in adult birds is influenced by several factors including the degree and type of contamination of the birds' overwintering and breeding-site foraging locations, composition of their diet, diet characteristics (energy density and proximate composition), foraging costs as well as species differences in chemical toxicokinetics such as metabolic biotransformation capacity and other mechanisms of chemical elimination (Focardi *et al.*, 1988; Mora, 1996; Frank *et al.*, 2001; Alcock *et al.*, 2002; Custer and Custer, 1995, Custer *et al.*, 2002; Custer and Read, 2006; Neigh *et al.*, 2006b; 2007). The time required for a bird's contaminant bioaccumulation to achieve steady state its environment and food varies tremendously for different organochlorine compounds, ranging from days for readily biotransformed chemicals to several years for recalcitrant, superhydrophobic compounds (Clark *et al.*, 1987; Drouillard *et al.*, 2001). Thus, individual contaminant loads in maternal tissues at

the time of egg formation reflect different time-integrated exposures of the adult bird, with rapidly cleared contaminants (short time to steady state) being mostly reflective of breeding site contamination, intermediate cleared chemicals potentially being more reflective of overwintering site contamination, and very slowly cleared chemicals reflecting time-weighted averaged concentrations at breeding and overwintering sites over a multi-year exposure period. Consequently, the study of contaminant signatures in migratory bird eggs is important in that it reveals the integrated contaminant conditions of their maternal resident locations and the breeding areas. Migratory birds are also thought to be sources of contaminant transport between different locations (Anthony *et al.*, 2007; Blais' *et al.*, 2007; Griffiths *et al.*, 2009).

We studied populations of three migratory bird species that breed in Point Pelee National Park of southwestern Ontario, Canada. 1) Tree swallows (*Tachycineta bicolor*; Hirundinidae) feed mainly on emergent aquatic insects. Previous studies found that the diet of tree swallows was predominantly made up of aerial insects of aquatic origin, accounting for over 80% of the diet (Smits *et al.*, 2000; Mengelkoch *et al.*, 2004). Populations from southern Ontario overwinter in Florida, Cuba and Honduras (Butler, 1988). 2) Purple martins (*Progne subis*; Hirundinidae) feed at a higher altitude on land than tree swallows, and mainly consume aerial insects of terrestrial origin (Sibley, 2001). Walsh (1978) reported that over 75% of their diet was composed of terrestrial insects. Purple martins spend their non-breeding season in eleven different South American countries (Hill, 2001). 3) House wrens (*Troglodytes aedon*; Troglodytidae) are insectivorous, but unlike tree swallows and purple martins, which capture insects during flight, wrens are gleaners, collecting insects from tree foliage. More than 90% of their diet was composed of terrestrial insects (Beal *et al.*, 1916, reported in Gross, 1948). Wrens from the Canadian breeding range migrate to the southern United States and to north and central Mexico for winter (Johnson, 1998; Taylor *et al.*, 1983).

Even though these birds lay eggs in Point Pelee National Park area, PCBs and other persistent pollutants accumulate partially from their pre-breeding feeding grounds. Organochlorines have been banned in Canada and the United States for 30 years. Organochlorine concentrations measured within birds have decreased since these chemicals were banned (Martin *et al.*, 2003; Braune, 2007). However, migratory birds

from Latin American countries are still exposed to elevated levels and that is a concern (Mora, 2008). Many organochlorine (OC) pesticides were used until 2000 in Mexico in agriculture and disease vector control (Alegria *et al.*, 2006; Wong *et al.*, 2008; 2009). Thus, it is important to know birds' OC contaminant, PCB accumulation and PCB congener composition before the nestlings or adults of these species are used for biomonitoring studies.

The objective of this study was to determine whether the concentrations of PCB and other OC contaminants in the eggs differ among species and to determine if there is a difference in chemical signatures among species that is indicative of overwinter and breeding site exposures and/or differences in species specific foraging patterns. This study was also intended to provide information on maternal contributions of contaminants to juvenile birds in order to partition maternally derived and dietary sources in juveniles from the breeding site used in biomonitoring studies (Chapter 4).

Materials and methods

Point Pelee National Park is located at the southernmost tip of the Canadian mainland. The park's area is only 20 km² but its landscape is a mixture of marshes, forests, fields and beaches (Stewart, 1977; Parks Canada, 2003). The park provides suitable feeding and breeding grounds for many birds, and is an important staging area for spring and fall migrations of birds crossing Lake Erie. Point Pelee is located in the overlap path of the Atlantic and Mississippi flyways, which are important continental migratory routes (Proctor and Lynch 1993; Fletcher, 1997). All three bird species were studied in Point Pelee National Park and adjacent areas.

Twenty-one tree swallow boxes were placed in Point Pelee National Park in spring 2005 as part of a feeding experiment to study PCB bioaccumulation by tree swallow nestlings. They were located near the Marsh Boardwalk and adjacent beach area of the park. Each nest box was checked 3-4 times per week during which the stages of nest building and the egg laying dates were recorded. The tree swallow eggs were collected within 48 h of being laid. During the experiments the tree swallow nestlings were preyed upon, resulting in 100% mortality. The boxes were subsequently occupied by house wrens, whose eggs were also sampled from those boxes. Purple martin eggs were collected from just outside the park's northern boundary from colonies established

at Erie Shores Golf and Country Club in 2004. Because of severe predation in the park, only the minimum number of eggs were collected for this study.

In total, 17 eggs were collected - 5 eggs from purple martins, 4 from tree swallows, and 8 from house wrens. The eggs were collected from different clutches. As house wren eggs are very small, 2 eggs were pooled to make one sample. All eggs were weighed and stored at -20°C until chemical analyses were performed. The eggs that were left in the nests were weighed on the day of laying, and then observed until hatching to monitor population-specific hatching success.

Chemical analysis

The eggshells were removed and the egg yolk and albumen were used for analysis following the method of Drouillard and Norstrom (2001). Extracted egg contents ranged from 0.63 ± 0.07 g for house wren eggs, 2.38 ± 0.21 g for purple martin eggs and 1.26 ± 0.14 g for tree swallow eggs. Samples were dried separately by mixing in 35 g of sodium sulphate (VWR, Mississauga, ON) in previously cleaned and hexane rinsed mortars and pestles. Each sample was transferred to a glass column for solid-liquid extraction with 50 mL of dichloromethane (DCM): hexane (1:1; Fisher Scientific, Ottawa, ON) and spiked with 100 μL of a surrogate standard (a mixture of C^{13} labelled PCB 37, PCB 52 and PCB153 of concentration 200 ng/mL) to determine the recovery rate. An additional 250 mL of the same mixture was added to each column and allowed to stand for one h. Subsequently, each column was eluted, the extracts were rotoevaporated, and 10% of each sample was used for neutral lipid determination by drying in tinfoil plates in an oven and determining the mass of remaining lipids (Drouillard *et al.*, 2004).

Additional cleaning was done using gel permeation chromatography (GPC) to remove excess lipids and Florisil chromatography to separate different organochlorine fractions. The GPC column, containing SX-3 biobeads (BioRad, Mississauga, ON) and 50:50 DCM:hexane facilitated separation of lipids from organochlorines. The Florisil column contained 8 g of 60-100 mesh florisil (VWR, Mississauga, ON) wet packed in hexanes. Three different solvents (different ratios of DCM and hexane) were poured through the Florisil column and collected individual fractions. The first fraction consisted of 50 mL of hexane and was used to elute PCBs (including mono-ortho substituted congeners), chlorobenzenes, octachlorostyrene, trans-nonachlor, pp'-DDE, mirex and

photomirex. A second fraction (50 mL of 15:85% DCM:hexane) was used to elute, chlordanes, hexachlorocyclohexanes, cis-nonachlor, pp'-DDD and remaining -pp'-DDT. The third solvent fraction (130 mL of 60:40% DCM:hexane) eluted heptachloroepoxide and dieldrin.

PCB and other organochlorine analyses were completed by using a Hewlett-Packard 5890 gas chromatograph equipped with a 5973 mass selective detector (MSD) and a 5973 autosampler with a DB -5 column (Chromatographic Specialities, Brockville, ON, Canada). The carrier gas used was high purity helium and the injections were made in splitless mode. The injection volume was 2 μ L. The oven was programmed for 3 min. at 90⁰ C then allowed a gradient ramp of temperatures from 7 °C/min. to 150 °C and again another gradient ramp of temperature 3 °C/min. to a higher temperature of 280 °C and held for 5.1 min. MSDCHEM software was used to identify and integrate the different PCB congeners and other organochlorines based on their retention time, major ion and referenced against working standards diluted from certified standards (Accustandard Quebec Ministry of Environment PCB Congener Mixture and Accustandard Custom Organochlorine Pesticide mixtures).

Analytical accuracy was determined by the spiked surrogate standard's recovery. A blank and a standard homogenate sample (in house Detroit River fish homogenate) were analysed together with each five sets of samples. Recovery rates for different samples ranged from 65% to 135%. The concentrations ng/g wet weight (mean \pm SD) of PCB congeners 52 (72.45 \pm 24.85), 138 (276.76 \pm 82.59), 153 (568.53 \pm 185.61), 180 (472.97 \pm 134.17) and 194 (85.45 \pm 24.97) of in house reference homogenate were in the accepted range of the laboratory control chart values (mean \pm 3 standard deviation units).

Data analysis

All organochlorines including PCB congeners were expressed in concentration units of ng/g lipid. Σ PCB concentration was calculated as the sum of the concentrations of the 38 congeners (IUPAC #: 18, 17, 31/28, 33, 52, 49, 44, 74, 70, 95, 101, 99, 87, 110, 82, 118, 105, 151, 149, 153/132, 138, 158, 128, 156, 187, 183, 177, 171, 180, 191, 170, 201, 195, 194, 205, 208, 206 and 209x) analyzed and quantified in different egg samples. Σ DDTs refers to the sum of p,p'-DDT, p,p'-DDE and p,p'-DDD. Chemicals whose

concentrations were below detection limit were considered to be zero for the purpose of statistical analysis. All analyses were conducted using Statistica[®] version 6.1 software (Statistica, 1997). Analysis of Variance (ANOVA) was used to test for significance of differences among concentrations of Σ PCB congeners across species. Similar tests were performed with other OCs. Pairwise comparisons among means were performed using the Fisher LSD (least square difference) method at $p < 0.05$.

The chemical signatures measured in eggs were also compared using Principal Component Analysis (PCA). This technique is widely used as a data reduction method when numerous dependent variables are measured for a set of treatments (Mora, 1996). As the variances in some groups were not homogeneous, all data sets were \log_{10} transformed before statistical analysis. Chemicals detected in at least two eggs were included in the analysis.

Chemicals were also pre-categorized based on expected persistence patterns in avians according to known elimination rates and time to steady state measured for other species. Rapidly cleared chemicals (half-lives 10-60 d), intermediate cleared (>60-150 d); slowly cleared chemicals (half-lives > 150 d). They were categorized as follows:

Rapidly Cleared: OCS, dieldrin, PCB 18, 17, 31/28, 52, 49, 44, 70/76, 87, 95, 101, 110, 149, 151, 174

Intermediate: HCB, PCB 74,

Slowly Cleared: oxychlordan, mirex, DDE, PCB 85, 99, 105, 118, 128, 130, 137, 138, 146, 153/132, 156, 158, 171, 177, 178, 180, 183, 187/182, 195, 194, 200, 201, 203, 206, 209 (Clark *et al.*, 1987; Drouillard *et al.*, 2001; 2007).

Results

The clutch size, mass of the eggs and percent lipid all fell within the normal range for each species (Brown, 1997; Johnson, 1998; Robertson *et al.*, 1992) (Table 1). House wren clutches studied were the second brood (Johnson, 1998), as egg-laying began after midJuly. There was no significant difference in the lipid content of the eggs of three species ($F(2,10)=0.44$, $p > 0.05$). Moisture content of the eggs was not determined due to their small size.

Concentrations of OC contaminants and PCB congeners are reported on a lipid-normalized basis (Tables 2 and 3). Selected organochlorine pesticide chemicals from each of the three clearance categories (rapid, intermediate and slowly cleared) were examined for differences between species. The industrial byproduct octachlorostyrene (OCS) (identified as rapidly cleared) exhibited no significant differences (ANOVA, $F(2,10) = 2.1$, $p > 0.05$) in lipid normalized egg concentration among the three species. Hexachlorobenzene (HCB), categorized as one of the intermediate clearance compounds, demonstrated significant differences among three species (ANOVA, $F(2,10) = 7.36$, $P < 0.01$) with respect to lipid normalized egg concentrations. Compared to tree swallows and purple martins, house wren eggs had low values and it was significantly different from other two species (house wren vs. purple martin eggs $p < 0.01$; Fisher LSD and house wren vs. tree swallow eggs $p < 0.004$; Fisher LSD). The eggs of tree swallows and purple martins showed no significant differences ($p > 0.3$; Fisher LSD).

Mirex was chosen as a representative of slowly cleared insecticide. The lipid normalized concentration of mirex in eggs showed significant differences across species (ANOVA, $F(2, 10) = 12.31$, $p < 0.002$). Between species, egg mirex concentrations was significantly higher in tree swallow eggs (tree swallow vs. house wren $p < 0.001$; Fisher LSD and tree swallow vs. purple martin $p < 0.001$; Fisher LSD). There was no significant difference in the concentration of mirex in the eggs of house wrens and purple martin eggs ($p > 0.9$; Fisher LSD). Figure 1 shows the differences among species in the distribution of OCS, HCB and mirex.

In addition to the clearance categories above, contrasts were made between the species differences in bioaccumulation of Σ DDT and Σ PCBs since these two chemicals are known to locally track terrestrial versus aquatic exposure sources within the park. Historic uses of DDT at Point Pelee National Park resulted in enriched concentrations of this compound and its metabolite in soils of the park and has been used as a general marker of terrestrial feeding whereas PCBs tend to exhibit higher concentrations in aquatic insects (Smits *et al.*, 2005). Tissue analyses of some park species have shown high levels of DDT (Natural Heritage, Soil Contamination Study, 2001). Analysis of soil samples of the park by the same study has revealed some areas where DDT levels are above recommended government agency guidelines. There was heavy use of DDT in the

park by farmers during 1950s and 1960s to control agricultural pests and biting insects. In the 1970s DDT use was banned from the park, but still the levels of DDT are higher in the park environment than government guidelines. Depending on location and different sources Lake Erie sediments have varied levels of PCBs (Gewurtz and Diamond, 2003; Marvin *et al.*, 2004; Heidtke *et al.*, 2006). The immature stages of most aquatic insect species in the park spent most of their larval life burrowing and feeding in the sediments.

The Σ DDT lipid normalized egg concentrations were observed to be significantly different across species (ANOVA, $F(2, 10) = 12.7, p < 0.002$). Between the species, Σ DDT concentrations were higher in tree swallow eggs than in the other two species (between tree swallow and house wren ($p < 0.02$; Fisher LSD) and between tree swallow and purple martin ($p < 0.0005$; Fisher LSD)). There was no difference between purple martin and house wren eggs ($p > 0.05$; Fisher LSD). There were highly significant differences (ANOVA, $F(2, 19) = 74.1, p < 0.0001$) among Σ PCB concentrations in eggs among species. For this class of compounds, there was no significant difference among Σ PCB concentration between the eggs of house wren and tree swallows ($p > 0.38$; Fisher LSD), but both species were higher than purple martin eggs (purple martin vs. house wren $p < 0.0001$, Fisher LSD and purple martin vs. tree swallows $p < 0.0002$; Fisher LSD). Figure 2 summarizes among species differences in Σ PCBs and Σ DDTs concentrations in eggs.

The PCA analysis indicated that first three factors explained 73% of the variation among eggs, with 65% of the variation being associated with PC factors 1 and 2. Factor loadings of different PCB congeners and organochlorine pesticides are summarized in Table 4. The first PC axis was strongly associated with highly chlorinated PCB congeners starting from penta-chlorinated congeners and dominated by congeners with no open meta-para positions in their biphenyl ring, previously categorized as slowly cleared compounds. Among the organochlorine pesticides, only p,p'-DDE and p,p'-DDT strongly loaded to the first axis. All chemicals loadings on the first PC axis were categorized as slowly cleared chemicals. PCB 149 was an exception, which exhibited a high loading to axis 1 but was classified as rapidly cleared. The second PC axis showed high loadings for less highly chlorinated congeners - mainly tri to penta-chlorinated congeners with low K_{ow} values and included congeners susceptible to biotransformation

and rapid elimination including PCBs 31/28, 52, 70, 95, 151 (readily cleared congeners). Among the organochlorine compounds, the PC 2 axis was strongly associated with chlordanes (cis and trans-chlordane and mirex). Most of the chemicals associated with PC 2 were categorized as rapidly cleared chemicals except for mirex which is slowly eliminated. Factor 3 loadings (not shown) tended to exhibit high loadings for QCB, HCB, OCS and p,p'-DDD). There was not an identifiable trend among chemicals associated with this axis and chemical persistence categories.

The ordinations of each egg based on factor scores on the first two PC axes are presented in Figure 4. The graphic representation demonstrates clear differences in the organochlorine chemical signatures across species. House wren eggs and tree swallow eggs were overlapping on the first factor plane and were distinctly different from purple martins. Whereas on factor two purple martins and house wrens were overlapping in their score values but tree swallows were distinctly different in their placement. House wren eggs and tree swallow eggs had high amounts of persistent congeners while purple martins were having lower concentration of these compounds (Figure 5). Tree swallows were remarkable in their higher accumulation of readily cleared PCBs and mirex, while purple martins and house wrens contained lower amounts of these compounds. Purple martin eggs showed more inter-egg variation on both planes than observed for the other two species.

Discussion

OC contaminants

Of the 19 OC compounds analysed, DDTs, mirex, HCB, OCS, β BHC, and heptachlor epoxide were detected in all eggs. Industrial byproducts 1245 tetrachlorobenzene (1245-TCB) and 1234 tetrachlorobenzene (1234-TCB) were not detected in any egg. HCB, a fungicide and an industrial byproduct banned in Canada since 1970 was detected in all eggs, whereas pentachlorobenzene (QCB), another industrial byproduct was found in all tree swallow eggs but not detected in some wrens and purple martin eggs. Components of the insecticide lindane (alpha, beta, gamma and delta-hexachlorocyclohexane; α -HCH, β -HCH and γ -HCH) were detected in low

Table 1. (Mean \pm SE) egg weight (g wet mass), clutch size (eggs) and lipid (percent) in three species

Species	Egg weight (g)	Clutch size	Lipid (%)
House wren	1.57 \pm 0.27 (n=24)	5.54 \pm 0.75 (n=13)	12.2 \pm 2 (n=4)
Purple martin	4.17 \pm 0 .27 (n=29)	4.35 \pm 0.87 (n=26)	12.4 \pm 5.7 (n=5)
Tree swallow	1.7 \pm 0.24 (n=38)	6.06 \pm 0.64 (n=9)	9.8 \pm 1.8 (n=4)

Table 2. (Mean±SE) organochlorines ng/g lipid in the eggs of three species

	House wren	Purple martin	Tree swallow
Compound			
1245-TCB	ND*	ND	ND
1234-TCB	ND	ND	ND
QCB	1.6±0.79	5.55±3.05	4.83±2.3
HCB	10.02±3.9	24.16±8.03	38.57±17.04
a-BHC	8.17±4.45	15.32±10.53	26.4±13.74
b-BHC	35.84±14.61	82.8±73.18	90.19±83.92
g-BHC	86.54±51.81	120.66±134.06	50.15±25.09
OCS	15.31±4.29	26.53±16.01	36.55±11.03
Heptachlor Epoxide	27.12±12.7	196.69±96.02	115.02±34.24
Oxychlordane	38.61±15.18	60.79±37.76	114.36±2.59
trans-chlordane	ND	2.97±1.19	28.52±23.51
cis-chlordane	3.04±1.51	44.49±27.44	79.92±40.98
trans nonachlor	4.51±2.21	13.76±7.63	34.89±6.54
p,p'-DDE	2670.73±921.64	1382.18±504.63	6664.06±2181.72
Dieldrin	134.89±66.08	55.49±22.2	193.64±105.28
p,p'-DDD	18.15±8.82	92.65±148.21	118.36±60.42
cis-nonachlor	4.44±2.17	5.36±2.9	16.97±5.02
p,p'-DDT	177.96±118.21	61.73±41.73	144.3±73.42
Mirex	21.02±10.26	27.96±32.55	169.18±62.02

*ND Not Detected

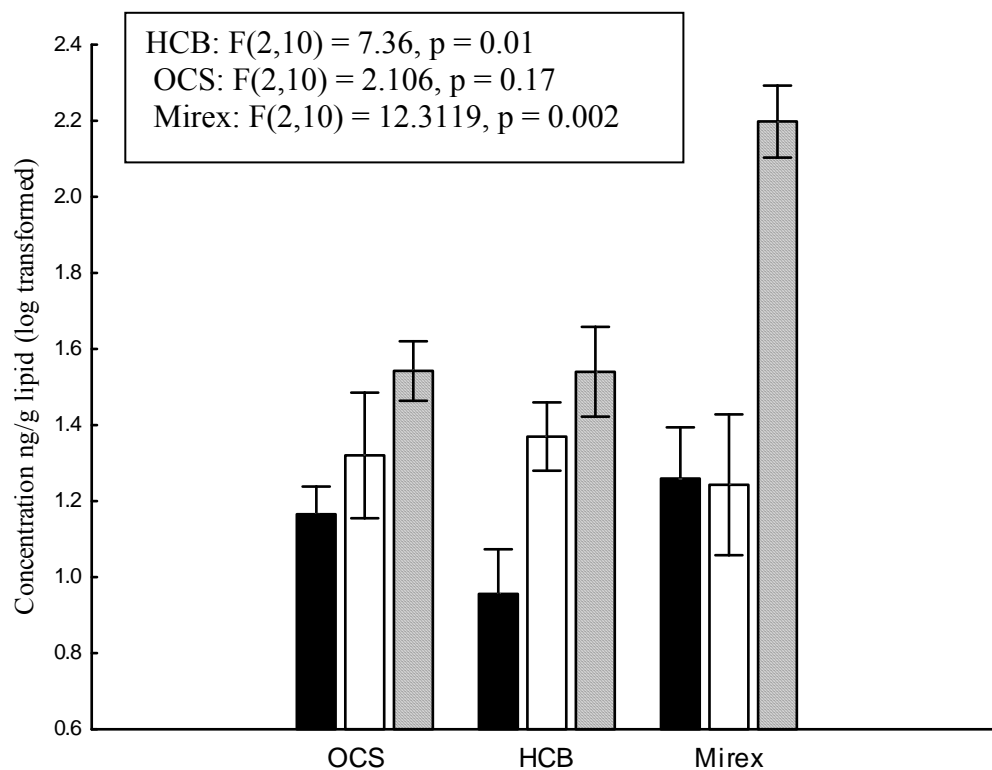


Figure 1. (Mean \pm SE) concentrations of OCS, HCB and mirex in eggs of three species (black bars –house wren eggs n=4, white bars – purple martin eggs n=5, and grey bars – tree swallow eggs n=4)

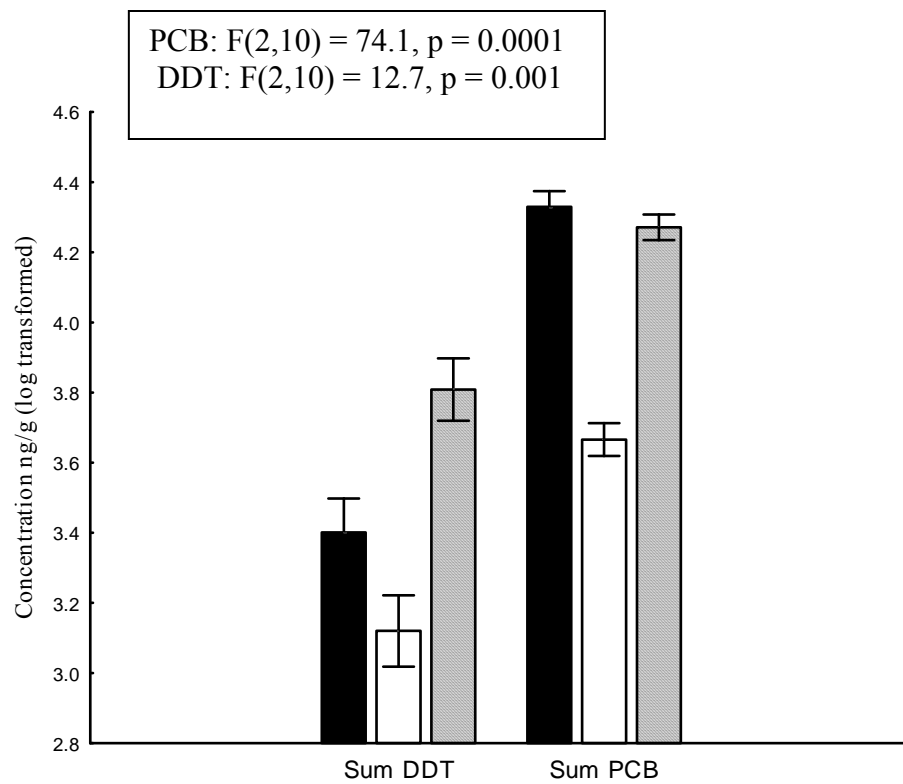


Figure 2. (Mean \pm SE) concentrations of \sum PCB and \sum DDT (black bars - house wren $n=4$, white bars - purple martin $n=5$, and grey bars - tree swallow $n=4$)

Table 3. Mean \pm SE PCB congeners ng/g lipid in the eggs of three species

PCB congener	House wren	Purple martin	Tree swallow
PCB 18	0.85 \pm 0.86	0.82 \pm 0.5	1.94 \pm 1.61
PCB 17	ND	0.28 \pm 0.43	ND
PCB 31/28	16.01 \pm 8.69	13.48 \pm 8.08	47.33 \pm 3.23
PCB 33	ND	ND	ND
PCB 52	6.51 \pm 1.5	15.56 \pm 7.72	98.28 \pm 8.8
PCB 49	15.12 \pm 9.11	12.69 \pm 6.02	33.04 \pm 3.45
PCB 44	2.38 \pm 0.27	0.15 \pm 0.3	4.99 \pm 2.46
PCB 74	118.54 \pm 32.65	25.98 \pm 9.21	77.18 \pm 4.86
PCB 70	11.23 \pm 2.71	22.23 \pm 7.9	64.93 \pm 8.37
PCB 95	9.92 \pm 3.2	23.18 \pm 6.24	94.69 \pm 7.18
PCB 101	251.17 \pm 150.72	117.31 \pm 38.44	299.93 \pm 20.9
PCB 99	368.76 \pm 73.18	79.25 \pm 24.38	176.06 \pm 11.56
PCB 87	107.65 \pm 33.38	32.89 \pm 14.58	89.15 \pm 7.69
PCB 110	189.72 \pm 122.5	95.47 \pm 21.78	276 \pm 27.82
PCB 82	ND	ND	ND
PCB 118	1044.74 \pm 122.53	176.2 \pm 47.2	607.9 \pm 44.05
PCB 105	389.41 \pm 47.37	63.32 \pm 20.32	266.32 \pm 22.72
PCB 151	24.1 \pm 12.07	39.91 \pm 8.77	111.46 \pm 7.08
PCB 149	460.02 \pm 166.37	190.79 \pm 52.33	600.89 \pm 47.28
PCB 153/132	4113.14 \pm 747.08	810.61 \pm 176.46	2854.14 \pm 239.26
PCB 138	2936.53 \pm 581.87	675.44 \pm 144.42	2523.98 \pm 286.81
PCB 158	198.19 \pm 24.39	59.73 \pm 32.24	169.26 \pm 23.31
PCB 128	385.82 \pm 66.09	84.83 \pm 15.96	302.42 \pm 41.98
PCB 156	288.05 \pm 39.64	47.23 \pm 13.79	194.73 \pm 22.79
PCB 187	1267.91 \pm 207.4	289.89 \pm 74.89	1306.7 \pm 179.14
PCB 183	642.41 \pm 95.1	164.58 \pm 45.64	598.58 \pm 62.93
PCB 177	365.27 \pm 44.75	109.85 \pm 35.48	384.48 \pm 83.71
PCB 171	427.65 \pm 252.38	50.27 \pm 17.25	190.07 \pm 49.94
PCB 180	3935.49 \pm 633.07	727.06 \pm 174.6	3530.35 \pm 463.74
PCB 191	ND	ND	ND
PCB 170	1608.57 \pm 238.4	322.8 \pm 76.12	1458.48 \pm 226.96

Table 3 (Cont'd). Mean± SE PCB congeners ng/g lipid in the eggs of three species (contd.)

PCB congener	House wren	Purple martin	Tree swallow
PCB 201	709.08±110.3	181.26±39.89	749.84±143.49
PCB 195	209.59±24.39	62.27±13.81	235.12±25.24
PCB 194	1228.64±262.77	215.41±50.21	1143.12±345.93
PCB 205	15.39±14.54	ND	61.11±30.06
PCB 208	64.4±15.72	20.58±3	64.36±6.72
PCB 206	317.33±84.58	17.23±34.46	285.43±57.33

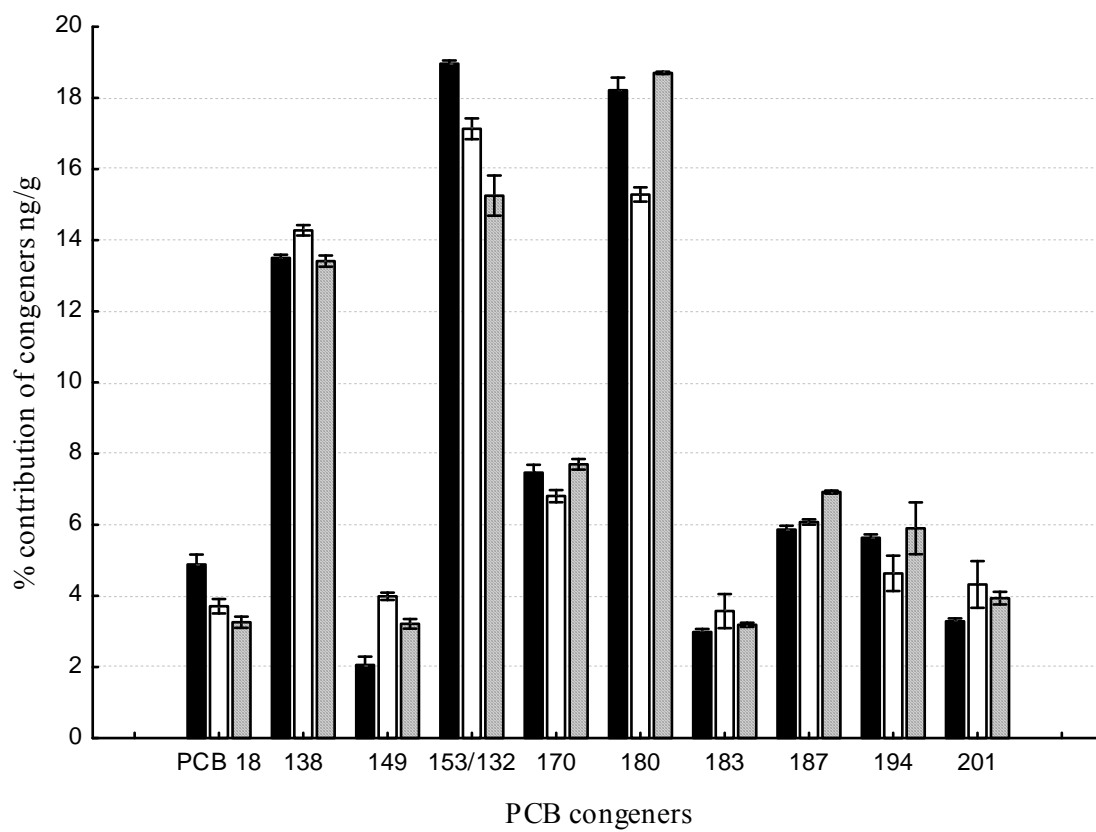


Figure 3. Mean \pm SE % contribution of ten most common PCB congeners in the eggs (black bars - house wren n=4, white bars - purple martin n=5, and grey bars - tree swallow n=4).

Table 4. Factor loadings (varimax raw) of the eggs of three species (Extraction: Principal components)

Variable	Factor 1	Factor 2	Variable	Factor 1	Factor 2
QCB	-0.10	0.35	PCB 99	0.96	-0.19
HCB	-0.18	0.43	PCB 87	0.81	0.01
a-BHC	-0.09	0.07	PCB 110	0.70	0.35
b-BHC	-0.36	-0.06	PCB 118	0.98	-0.06
g-BHC	-0.13	-0.06	PCB 105	0.96	0.01
OCS	0.07	0.19	PCB 151	-0.21	0.83
Heptachlor Epoxide	-0.51	0.28	PCB 149	0.91	0.30
Oxychlordan	0.36	0.48	PCB 153/132	1.00	0.00
trans-chlordane	0.07	0.49	PCB 138	0.99	0.08
cis-chlordane	-0.15	0.22	PCB 158	0.95	0.05
trans nonachlor	0.11	0.93	PCB 128	1.00	0.02
p,p'-DDE	0.66	0.40	PCB 156	0.96	-0.02
dieldrin	0.18	0.27	PCB 187	0.98	0.18
p,p'-DDD	-0.07	0.31	PCB 183	0.98	0.11
cis-nonachlor	0.02	0.93	PCB 177	0.97	0.17
p,p'-DDT	0.69	-0.11	PCB 171	0.93	-0.13
mirex	0.30	0.90	PCB 180	0.99	0.12
PCB 18	0.26	0.22	PCB 170	0.99	0.12
PCB 17	-0.03	-0.03	PCB 201	0.98	0.17
PCB 31/28	0.55	0.60	PCB 195	0.98	0.21
PCB 52	0.15	0.94	PCB 194	0.95	0.09
PCB 49	0.53	0.52	PCB 205	0.39	0.22
PCB 44	0.58	0.09	PCB 208	0.97	0.07
PCB 74	0.94	-0.08	PCB 206	0.87	0.23
PCB 70	0.09	0.92	Expl.Var	24.17	8.47
PCB 95	0.06	0.93	Prp.Totl	0.47	0.17
PCB 101	0.78	0.27			

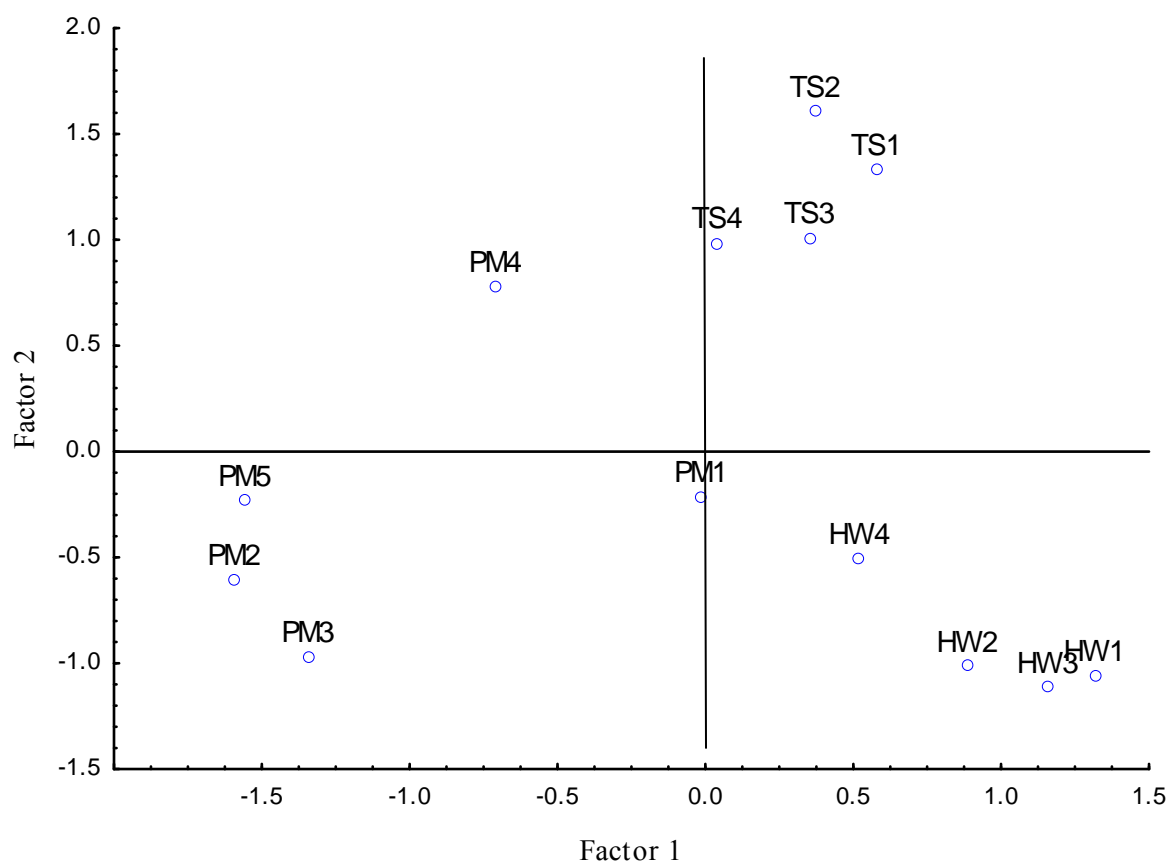


Figure 4. Plot of principal components 1 and 2 based on the contribution of different PCB congeners and other organochlorines in the eggs of three species

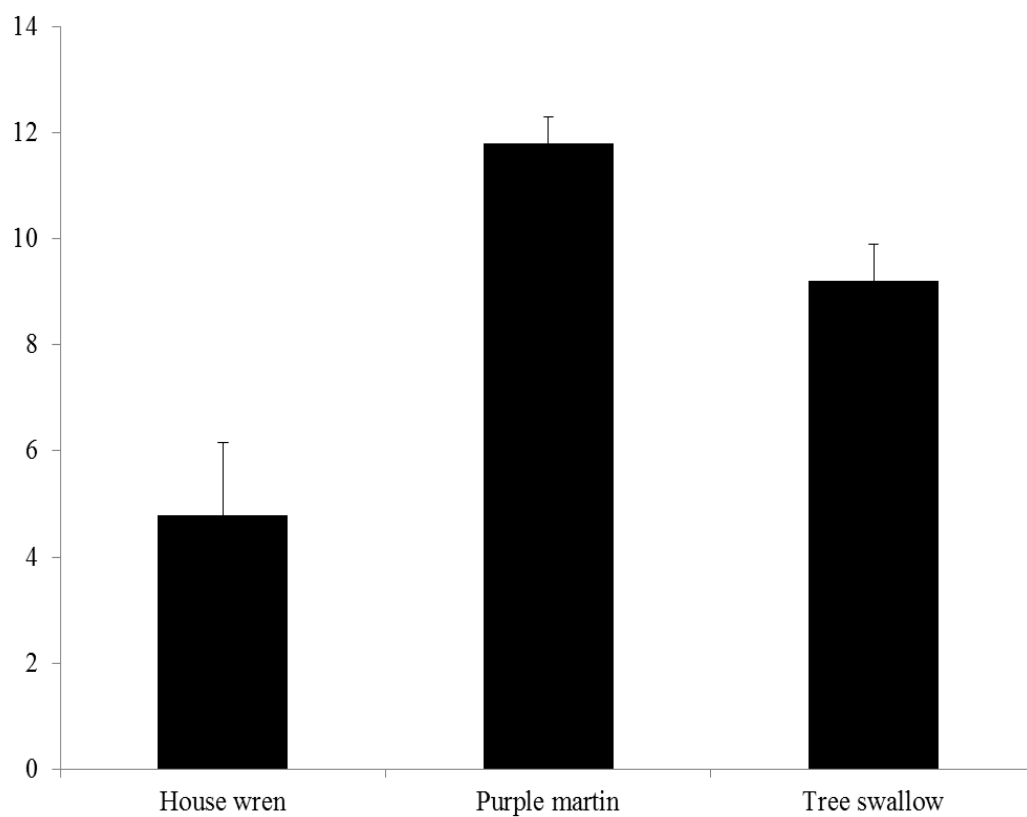


Figure5. (Mean \pm SE) % concentration of readily cleared PCB congeners in three species

concentrations in most of the eggs all three species. Insecticides trans-chlordane and cis-chlordane and their metabolites oxychlordane, trans nonachlor, cis-nonachlor and heptachlor epoxide were also detected in most of the eggs. DDT and its metabolites were detected in all eggs. The metabolite in greatest concentration was p,p'-DDE and it is the most persistent of all three. This compound was found in highest concentrations in tree swallow eggs. Levels of DDE that can induce behavioural changes (unattentiveness by parents - 2,8 µg/g; DeWeese *et al.*, 1985) were ten times higher than what was observed in house wren eggs (0.28 µg/g wet weight - this study), and four times higher than what was present in tree swallow eggs (0.62 µg/g wet weight=this study). The concentrations of p,p'-DDE in all eggs examined were below the 'no observable adverse effect level (NOAEL)' of 2.2 µg/g reported for terrestrial birds by Clarks *et al.* (1995) and also lower than established NOAEL and 'lowest observable adverse effect level (LOAEL)' values reported for p,p'-DDE in bird eggs by other investigators (Elliot and Harris, 2002; Anthony *et al.*, 2007; Hernández *et al.*, 2008). DeWeese *et al.*, (1985) found tree swallow eggs that were unattended by parents had total DDT of 2.8 mg/kg, which is far above the values we measured in our tree swallow eggs. We didn't notice any behavioural changes in the parents in the field during our study period. The same study by DeWeese *et al.* (1985) again pointed out that the DDE threshold for reproductive effects on passerine birds is about 8-9 mg/kg, which is very high compared to the values we found in the eggs in our study.

Mirex, an insecticide that was never used in Canada, was detected in all eggs. The concentration of mirex found in this study were much lower in tree swallow eggs when compared to 40.1 ng/g reported by Bishop *et al.* (1995) for the same species. They found mirex in 30% of the sediment samples they assessed along the St. Lawrence River Basin. The sediment sites were recorded as the area from which the nestlings accumulated their contaminants. The insecticide dieldrin was detected in at least one egg from each species in this study. Heptachlor epoxide, a metabolite of the insecticide heptachlor was found in all eggs of all three species.

Organochlorine contaminant concentrations of some chemicals observed in this study were greater than the concentrations reported in a tree swallow study by Custer *et al.*

(2002). Tree swallow eggs in their study from Wisconsin River, Wisconsin USA showed a heptachlor epoxide concentration of 0.009 µg/g (geometric mean) which was lower than our value of 0.011 µg/g wet weight. The same differences were noticed in the concentration of other contaminants like mirex 0.008 µg (geometric mean), oxychlordan 0.013 µg/g and p,p'-DDD and p,p'-DDE 0.099 µg/g and 0.002 µg/g, respectively. Concentrations of these contaminants were increased threefold in tree swallow eggs from our study. Shaw (1983) reported the concentration of DDE in viable and non-viable tree swallow eggs in central Alberta and found DDE concentrations varied from 1 µg/g wet weight to 2.5 µg/g wet weight and were higher than we measured in our study. We observed normal hatchability in the Point Pelee swallow eggs despite the presence of many OCs in the eggs (Smits *et al.*, 2005).

ΣPCB and congeners

Differences in the total number of congeners included in different studies makes it difficult to compare ΣPCB across studies. Most bird species accumulate common congeners in larger proportions compared with other species (Custer and Read, 2006; Antoniadou *et al.*, 2007). These common congeners allow us to make comparisons across species. Of the 38 congeners analysed in this study, 10 made up 2% or more of the total (Fig. 3). Three congeners, IUPAC numbers 153/132, 180 and 138 each represented more than 10% of the total. These same congeners were reported as most abundant in tree swallows from the Housatonic River watershed, western Massachusetts, USA, by Custer and Read (2006). They reported high concentrations of congeners with Ballschmiter and Zell (BZ) numbers: 153, 138, 180, 187, 149, 101, and 170. In our study, each of those congeners 153/132, 138, 187, 180, 170 and 194 represented more than 5% of the total congeners analyzed (Figure 3). These congeners also dominated in eggs of colonial waterbirds from Galveston Bay and east Texas, USA (Frank *et al.*, 2001) and in eggs of waterbirds from Lake Kerkini, a northeastern Mediterranean wetland (Antoniadou *et al.*, 2007). Similarity of common congeners found in bird eggs in our study with those in other studies suggests that the levels of persistent congeners show a global level distribution and clearance/elimination pattern (Alcock *et al.*, 2002). All congeners represented above except PCB 101 and 149 are slowly cleared congeners. It shows that all these congeners share their property of persistence in all

media including animal tissues.

Levels of PCB concentration found in the eggs of all three species were not elevated to a level to show embryotoxicity or unhatchability. A study by Barron *et al.*, (1995) reported that a concentration of 3.1 µg/kg of PCB126 brought embryo mortality in chicken eggs but the value for the same effect in double cormorant eggs was 30 mg/kg ΣPCB. Different species need different concentrations of PCB to develop sensitivity and characteristic effects (Barron *et al.*, 1995). LD 50 concentration for PCB 77 for chicken was 9 mg/kg, for pheasants it was more than 100 mg/kg and for mallards it was much higher with a value of more than 5000 mg/kg (Barron *et al.*, 1995). Hoffman *et al.*, (1998) experimentally demonstrated that air cell injections of PCB 126 caused malformations and edema in chickens at 0.3 ppb, 2.3-23 ppb in kestrels and in terns, hatchability was affected at 44 ppb. LD 50 for PCB 126 for chickens was suggested as 0.4 ppb, 65 ppb for kestrels and 104 ppb for terns.

For *ortho* PCBs, concentrations above 20 µg/g wet weight were reported to cause reduced hatching, embryo mortality, and deformities in birds (Gómara *et al.*, 2008). The threshold for potential reduction in hatchability for PCB 77 was reported as 1 µg/g; however, one mourning dove egg with higher concentrations show no effect (García-Hernández *et al.*, 2006). None of the congeners analyzed in our study exceeded the value of 1µg/g wet weight. We observed normal hatchability for all three species and no noticeable deformities in nestlings hatched from sibling eggs.

Site of contaminant accumulation

Principal component analysis was used as a data reduction method to better establish pattern differences in contaminant signatures of eggs between the species and relate these patterns to chemical persistence/clearance categories. The differences in chemical profiles among species suggests differences in overall exposures by species that could be related to both differences in breeding site feeding ecology as well as differences in the magnitude of environmental residues at over-wintering stations.

Tree swallows

Tree swallow eggs were notably different from the other two species; they had higher levels of easily metabolizable, rapidly cleared, PCB congeners. This could be influenced by two competing hypotheses: 1) they are more recently exposed to readily cleared congeners at the breeding site and are approaching steady state with metabolized PCBs but not achieving steady state (i.e. a multi-year process) with persistent ones and/or 2) tree swallows have lower biotransformation capacity for readily cleared PCBs compared to other species of birds.

The diet of tree swallow differs from the other two species in that it includes a high composition of aquatic insects. A previous study by Smits *et al.*, (2005) showed that the diet of tree swallow nestlings had considerable amounts of PCBs (mainly from *Hexagenia* mayflies) that include both persistent and metabolized congeners according to their relative abundance in Aroclor mixtures. They found that aquatic insects at Point Pelee National Park tended to be enriched in PCBs compared to terrestrial insects. Smits *et al.*, (2005) further showed that the amount of mayflies included in the diet and the Σ PCB in tissues of tree swallow nestlings were positively correlated indicating that breeding site sources of PCBs are important contributors to the total exposures of these compounds in nestlings. The biotransformation capacity of tree swallows for rapidly cleared PCB congeners (i.e. those chemicals with vicinal hydrogen substituents at *meta-para* positions on one of the phenyl rings in the PCB molecule; Drouillard *et al.*, 2001), have not been directly characterized, but have often been assumed to be low for this species (Nichols *et al.*, 1995; 2004). Papp *et al.*, (2007) showed that the PCB congener pattern of nestling tree swallows was very close to the congener pattern of mayflies they consumed and concluded that tree swallow nestlings have low biotransformation capabilities. For other species of birds including American kestrels, juveniles were shown to have similar or higher PCB biotransformation rates compared to adults (Drouillard *et al.*, 2007). Thus, there appears to be some support for lower overall biotransformation capacities of tree swallows for readily cleared PCBs. However, in order to better distinguish the steady state/non-steady state hypothesis with metabolic biotransformation capacity, a different experimental design would be required whereby eggs from birds of different ages would need to be collected. In this case, an observation

of young, reproductive adult birds producing eggs with a higher relative abundance of readily cleared congeners and older birds producing eggs more depleted in these compounds would support the steady state/non-steady hypothesis. Further study would be necessary to test this but would require multi-year banding efforts at the colony, which is beyond the scope of this thesis.

Tree swallows were also found to be enriched in Σ DDT, and had some of the highest Σ DDT concentrations measured among the species of study. This observation is in apparent contrast with the chemical signatures present in the breeding site diets of these birds, which contain predominately aquatic insects that tend to have lower Σ DDT compared to terrestrial insects at the breeding ground (Smits *et al.*, 2005). Taken together, the two observations would appear to implicate that both over-wintering sources and breeding sources are contributing to the overall bioaccumulation of organochlorine contaminants in adult tree swallows. Higher mirex contamination of tree swallow eggs (PCA factor 2 loadings) may also be suggestive of breeding site exposures of this insecticide given its known use for fire ant control in the Southern United States (Kutz *et al.*, 1985). Mirex was reported to be present in the sediments of the Great Lakes region and was assessed to be a threat to higher trophic level organisms including birds (Bishop *et al.*, 1995; Apeti and Lauenstein, 2006).

House wrens

House wrens have the highest levels of PCBs categorized as persistent compared to all species. House wrens feed mainly on terrestrial insects (Skutch 1953; Neigh *et al.*, 2006a; Chapter 3). Gross (1948) cited different studies that indicted the preference of terrestrial insects by house wrens. Terrestrial insects in the park are depleted of PCBs compared to aquatic insects (Smits *et al.*, 2005; Chapter 3). The diet of wren nestlings composed primarily of terrestrial origin and which carry less PCB and the abundance of highly persistent PCB congeners in the eggs of this species make it clear that they assimilated PCBs from overwintering habitat with unknown PCB concentrations. Neigh *et al.*, (2006b) reported very high PCB concentration in a house wren egg (26 mg/kg wet weight). The concentrations of Σ PCB in nestlings were low compared to eggs in their study at Kalamazoo River (Michigan, USA). This difference in concentration of nestlings

compared to eggs, and the low PCB content in terrestrial insects suggest the wrens are heavily exposed to PCB at the overwintering sites. García-Hernández *et al.*, (2006) studied mourning dove and burrowing owls' eggs in the Colorado River Delta in Mexico and found higher concentrations of PCB 87 (mourning dove, a granivore 67 ± 71.5 (mean \pm SD) and in burrowing owl, carnivorous-insectivorous, 651.1 ± 644.8) PCB 158 (mourning dove 133.2 ± 261.3 ; burrowing owl 19.5 ± 18.6) and PCB 180 (mourning dove 39.4 ± 45.2 ; burrowing owl 16.12 ± 24.52) ng/g wet weight. One of the mourning dove eggs showed a very high concentration of PCB congeners (PCB 77 -1157ng/g; PCB 179- 7940 ng/g and PCB 158-1138 ng/g). Again, this suggests house wrens were exposed to high levels of PCB in their overwintering geographical range.

Purple martins

For purple martins, there is a consistent story of low exposures at both the overwintering and breeding grounds. Given the depleted DDT levels and terrestrial feeding, this suggests that overwintering sites may be of greater importance but that some individuals like PM1 and PM4 (Figure 4) that were shifted in their axis were more locally influenced. PM4 had a very high level of mirex (92.96 ng/gm) when compared to all other eggs (11.72 ± 1) and both PM1 and PM4 had higher proportion of at least two readily cleared congeners PCB 31/28 and PCB 49. The enrichment of these two readily cleared congeners suggests local accumulation. Like tree swallows, PM4 may be influenced by the mirex accumulation and were very closely placed to swallow eggs in the PCA factor planes based on its score on factor 2.

The overwintering locations of purple martins also influence their low levels in both ΣPCB and ΣDDT . Some of our data on Caribbean POPs indicates that S. America may be depleted in PCBs. This is consistent with their overwintering habits relative to tree swallows and house wrens where Mexico and the southern U.S. may have enriched contamination (Mora 1996; García-Hernández *et al.*, 2006).

Accumulation of other OCs

Presence of OCS, a readily cleared organochlorine industrial byproduct in all eggs suggests that they accumulated in the breeding site or may be accumulated at a very high amount at the overwintering location. There are not many studies showing the accumulation of these chemicals. Martin *et al.*, (2003) in the Great Lakes basin found OCS contaminant in osprey eggs from almost all locations of their study; nestlings from only one location had OCS in their tissues. Additionally, they found HCB in all eggs but not in all nestlings. HCB has an intermediate clearance rate and was found to be in lower concentrations in house wren eggs. This low concentration of OCs in wren eggs shows their accumulation from overwintering locations and higher levels of those chemicals in purple martin and swallow eggs show their local accumulation. Custer *et al.*, (2002) also reported the presence of HCB in very low amounts in the eggs of hooded mergansers, wood ducks and tree swallows from Wisconsin River, Wisconsin USA. The study suggests that these two contaminants can be accumulated both locally and also from overwintering locations.

It was expected that the distribution of pollutants in the eggs would be in a different order as per our understanding of the target insect orders in their diet from previous studies. Wrens were expected to have the highest burden of DDT and its metabolites, and tree swallows the least because aerial adults of aquatic insects accumulate less DDT than insects whose larvae are terrestrial herbivores, at least locally (Smits *et al.*, 2005). For the same reason, we expected PCB accumulation to be lowest in wrens and greatest in tree swallows. The egg data showed a different trend, possibly reflecting the pollution conditions of the overwintering sites of these passerines and their diet composition as mentioned above. Wrens are migrating to more northern ranges compared to the other two species. Detailed information on contaminant sources would depend on having precise knowledge of each population's overwintering distribution. The results of this study also illustrate the importance of knowing the egg composition before using the nestlings for biomonitoring studies.

Bird eggs are used in many studies as a nondestructive biomonitoring tool for persistent organochlorines (Van den Steen *et al.*, 2009). Tree swallow adults, and their eggs and nestlings are often used as a sentinel species to monitor the trophic transfer of

aquatic sediment bound pollutants (Smits *et al.*, 2000; 2005; Secord *et al.*, 1999). House wrens are used as biomonitors for terrestrial bound pollutants (Neigh, 2007). Even though all three species have overlapping breeding ranges, the profile of their pollutant burdens reflects both accumulation at different prebreeding locations and differences in their local diet pattern.

The amount of lipid passed from maternal tissue to the egg depends on many factors including clutch size, size of eggs and the mode of development - precocial or altricial (Drouillard and Norstrom, 2001). Van den Steen *et al.* (2009) suggested that because birds allocate large amounts of energy to egg production, maternal lipids should be replaced by dietary lipids. Consequently, the replacement clutch and second clutch will be mainly influenced by dietary lipids from breeding locations. In particular, we have observed wrens feeding on emergent adults of burrowing mayflies (Ephemeroptera: *Hexagenia*), and clutch initiation began after the peak emergence period of mayflies from Lake Erie. Smits *et al.* (2005) and Papp *et al.* (2007) reported a significant positive correlation between sum PCBs in the tissues of tree swallow nestlings and the amount of mayflies in their diet.

Eggs of these three passerine species breeding in Point Pelee National Park and adjacent areas have detectable contaminant burdens. The burdens exhibit a pattern that differs from expectations based on the proportions and composition of aquatic and terrestrial insect in their diets during the breeding season. As previously mentioned, tree swallow diets contain proportionately more aquatic insects, wrens are more terrestrially based, and purple martins are intermediate in diet. Smits *et al.* (2005) reported that swallow nestlings developing in the park and fed aquatic insects had more PCBs whereas those fed with terrestrial insects had greater burdens of DDT and its metabolites. Alegria *et al.* (2006) measured organochlorine pesticides in ambient air of Chiapas, Mexico during 2000-2001 and found elevated levels of DDTs, chlordanes and toxaphane compared with levels in the Great Lakes region. DDT was used in parts of Mexico and other Latin American countries until the year 2000 (although in restricted amounts), likely contributed to the bioaccumulation of these chemicals in insectivorous passerines.

Thus, it is essential to know the overwintering areas of these birds, their dietary habits and the contaminant burdens of the insects in their diet.

Even though all three species are insectivorous, the accumulation of pollutants depends on the diet, insects' trophic levels and trophic habitats (Bouwman, 2008). Insects captured from the same location can accumulate different contaminant loads based on diet. Therefore, differences in the trophic position of insects in the birds' diet, contribute to differential contamination patterns among the three species studied.

Avian species that occupy higher trophic positions generally accumulate more pollutants (Hothem *et al.*, 2006). Food quality and availability are important determinants of contaminant accumulation by birds and their eggs (Van den Steen *et al.*, 2009). When eggs are used for biomonitoring, it is important to know the clutch initiation dates of the first clutch and second clutches, the half lives of different contaminants etc., because this information is important in indicating the degree to which female birds are still retaining and transferring contaminants accumulated from their prebreeding grounds relative to contaminants derived by feeding at their local breeding locations. This is especially true of migratory birds. But a closer examination of the congener patterns in the eggs compels one to look more closely at the local level contaminant patterns and their dynamics too.

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Chapter 3: Insect composition and the accumulation of persistent organochlorine contaminants in diets of the nestlings of three passerine species at Point Pelee National Park, Ontario, Canada

Introduction

Insectivorous passerines that have limited home ranges are suitable biomonitors for organochlorine (OC) contamination (Harris and Elliot, 2000; Chu, *et al.*, 2003; Custer *et al.*, 2000; 2005; Neigh *et al.*, 2006; 2007; Spears *et al.*, 2008). They accumulate hydrophobic organic contaminants through trophic transfer (Borga *et al.*, 2001; Maul *et al.*, 2006; Smits *et al.*, 2005) and have been shown to be fairly resistant to certain contaminants (McCarthy and Secord, 1999). This property makes them useful in monitoring the uptake of environmental contaminants; however, their utility as bioindicators requires detailed knowledge of both their diet and contaminant content in diet items.

Tree swallows are a common insectivorous passerine species that breeds in Point Pelee National park. Various qualities of tree swallows make them good candidates for bioindicator studies. These include their readiness to accept nest boxes, their availability and small body size (Jones, 2003). Because tree swallows often forage over water and collect aerial aquatic insects, their nestlings are frequently used as bioindicators for the accumulation of persistent aquatic sediment bound organic pollutants (Bishop *et al.*, 1995, 1999, Custer *et al.*, 1998; 2000; 2003; 2005; Custer and Read, 2006; Froese *et al.*, 1998, Secord *et al.*, 1999; Smits *et al.*, 2000; 2005; Brasso and Cristol, 2008; Jayaraman *et al.*, 2009). Similarly, house wrens have been used as biomonitors for terrestrial soil bound pollutants (Neigh *et al.*, 2006; 2007). Purple martins are intermediate in their dietary use of terrestrial and aquatic insects and thus are likely to accumulate pollutants from both the habitats.

The objectives of this study were to determine: 1) if the relative proportions of aquatic vs. terrestrial insects in the nestling diets of the three species differ; 2) if there are compositional differences (insect groups) in the diet of tree swallow, purple martin and house wren nestlings; 3) the degree to which nestling diets vary between consecutive

years; 4) if there are differences in the total contaminant contents in the three species' diets with regard to Σ DDTs and Σ PCB including their congener specific accumulation.

These three passerine insectivorous species are syntopic but they are segregated into different trophic niches. Tree Swallows forage on insects of both aquatic and terrestrial origin while they fly (McCarthy and Winkler, 1999). Purple martins are also aerial insectivores. Purple martins tend to forage over terrestrial rather than wetland habitats, feeding on large swarms of terrestrial insects (Sibley, 2001). House wrens are insectivorous but unlike trees swallows, which capture insects during flight, they tend to be 'gleaners' i.e., they collect insects from the leaves. The feeding strategy of birds largely determines their exposure to environmental contaminants especially persistent OC compounds (Sagerup *et al.*, 2002; Neigh *et al.*, 2006; Dauve *et al.*, 2009).

Materials and methods

Collection of diet insects of tree swallows

Tree swallows were studied from April to August of 2002, 2003 and 2005. In 2002 and 2003, we studied them in nest boxes situated in several different locations of the park (two contaminated and two reference sites). The locations of these boxes were selected based on land use records (apple orchards) and soil contaminant reports (Natural Heritage, Soil Contamination Study, 2001). The two areas that we defined as 'contaminated' for our study were those at which OC levels exceeded Ontario Ministry of Environment guidelines. The two contaminated sites were around the DeLaurier house and orchards (DeLaurier site) and old Camp Henry area (Camp Henry site). One reference site encompassed the administrative building and golf course areas of the park (Administrative Building site). The second reference area was in the vicinity of the Marsh Boardwalk area (Marsh Boardwalk site). Both reference area sites had similar land use history. Fifty standard nest boxes were erected in the park in 2001 (Appendix Figures 1, 2, 3 and 4). These boxes were placed in those areas for a bioaccumulation study. Details of the study are included in Smits *et al.* (2005). Of these, 21 nest boxes were relocated to the marsh boardwalk and adjacent beach area in 2005 (Appendix Fig. 5) for a feeding experiment, and those boxes were used for collecting diets of swallow nestlings in 2005. These boxes were monitored for occupancy by birds and breeding activities.

We devised a ligature method to collect bolus samples from swallow nestlings. When the chicks were around 7 to 10 days old (optimum size to handle and adequate feeding activities), the young birds were collared around the neck just below the crop for up to 45 min. This prevented them from swallowing the food items provided by their parents. Food boluses were then removed from the crops of the nestlings using blunt forceps. In 2002 and 2003 the bolus samples were stored in 95% ethanol until identification.

Collection of diet insects of house wrens

To collect diet samples from wren nestlings, we used the same ligature method employed for tree swallows. Samples were collected in 2005 from the same boxes where swallow nestlings were studied in the same year. Diet samples were collected from 7 to 10 day old nestlings.

Collection of diet insects of purple martins

The ligature method was not successful with purple martins so we devised another method, the parent trapping method (Quinney and Ankney, 1985). The parents were trapped in their nest box when they came to the nest box to provision their nestlings. We collected the parent's catch from their beak. A variety of traps has been developed by different researchers to capture different types of birds from their nests and from their habitat. Some complicated traps are effective at capturing large cup nesting birds; others are suited to small cavity nesting birds. The traps consist of a dropping wire door (trap door) that closes with an electronic radio-release triggering mechanism that prevents the bird's escape (Mock *et al.*, 1999). In most cases the researchers use remote controls to operate trap doors; however, we developed our own trap mechanism with the help of Ms. Mary Wilson, the caretaker of the Purple Martin colony at Erie Shores Golf and Country Club, Leamington, ON, just outside the park. We detached one of the normal doors of the colony and replaced with a modified door, which could be closed by pulling a long, thin, attached thread. Most of the time we captured adults entering the nest with the food samples, but we could collect only very few samples directly from the parents' mouth. In most cases, they dropped the prey and we could collect the insects from the nest cups and surroundings. Sometimes we couldn't see any prey items, probably because the parents

may have swallowed the food themselves. Because it took time to bring the nest colony units down from the tall poles on which they were erected the trapped parents had adequate time to consume the prey items. Diet samples were collected from 8 to 14 day old nestlings in 2004.

The collected insects were weighed on the day of collection from 2004 onwards and were then stored frozen at -20°C until the individuals could be identified to orders/family in the laboratory and contaminant analyses were performed

Identification of insects in the boluses

Thawed samples were emptied into a petri dish and the individual insects were teased apart using a fine-tipped needle. Most insects were almost intact and thus we could identify all of them. Insects were identified to order/family using keys of Borror, Triplehorn and Johnson (1992), Borror and White (1970), and McAlpine *et al.* (1987). Insect prey items recovered from food boluses were classified as aquatic (having spent at least the larval stage in water – primarily Chironomidae and *Hexagenia* (Ephemeridae)), or terrestrial (all life stages spent on land). The few semi-aquatic invertebrates collected (those whose larvae develop in saturated soil) were included in the terrestrial category.

We couldn't use the insects collected from bolus samples of swallow nestlings for contaminant analyses in 2002 and 2003 because they had been preserved in alcohol. We used the bolus samples collected in 2005. Thirteen boluses were pooled to make a sample for analysis. The insects collected from house wrens and purple martins did not yield enough biomass for contaminant analysis, so we collected additional insects from the vicinity of nest boxes by various methods. These insects were added to the combined bolus samples in the same proportion as we had assessed in the diet samples collected from the nestlings. Aerial sweeping was used to collect diet insects of purple martins, and bush sweeping and hand-picking were used to collect diet insects of wrens. Insects collected were frozen en masse in hexane-rinsed glass jars. In the laboratory, they were thawed, sorted into taxonomic groups and separated into wren and purple martin diet groups. They were then weighed and refrozen in hexane-rinsed foil until analyzed. We

collected sufficient biomass to permit analysis of two samples for purple martins and three samples for house wrens.

Contaminant analysis

Contaminant analyses were performed in the Great Lakes Institute of Environmental Research (GLIER) analytical laboratory. Concentrations of 38 PCB congeners and 19 pesticides (1245-TCB, 1234-TCB, QCB, HCB, α -BHC, β -BHC, γ -BHC, OCS, heptachlor Epoxide, oxychlordane, trans-chlordane, cis-chlordane, trans nonachlor, p,p'-DDE, dieldrin, p,p'-DDD, cis-nonachlor, p,p'-DDT and mirex) were assessed in each diet sample (total 7 samples, house wrens 3, purple martins and tree swallows 2 each).

From the weighed samples, approximately 1 g of tissue was used for the determination of moisture content. The moisture content was determined by drying the sample at 110⁰ C for 24 h in a drying oven. The rest of the insect samples were homogenised and dried with 25 g of sodium sulphate (VWR, Mississauga, ON) in a hexane-rinsed mortar and pestle. We followed the method described in Drouillard *et al.*, (2007). The sample mixed with sodium sulphate was passed through a column of 275 mL of 1:1 dichloromethane (DCM): hexane mixture (Fisher Scientific, Ottawa, ON) and an additional 10 g of sodium sulphate. This process separated the organic contaminants and lipids from the samples, and they were collected in the liquid that flowed out of the column. This liquid was rotoevaporated to 10 mL volume. One mL (10%) was pipetted from the sample and was used for lipid determination (Drouillard *et al.* 2004). The lipid content was determined by drying the 1-mL sample collected in an aluminum boat for 1 h in a drying oven at 110 °C.

The lipid content in these samples were higher than acceptable for GC-MSD machine, so the lipids from samples were removed by performing gel permeation chromatography (GPC). The GPC column was packed with porous SX-3 biobeads (BioRad, Mississauga, ON) and the solvent used for separating lipids and organochlorines was 50:50 DCM: hexane. After GPC, the extracted liquid was passed through a column of packed florisil containing 8 g of 60-100 mesh florisil (VWR, Mississauga, ON) soaked in hexanes. To separate different organochlorines three combinations of solvents were poured through the column in sequence. The first solvent poured was 50 mL of hexane, which separated PCBs (including mono-ortho substituted

congeners), chlorobenzenes, octachlorostyrene, trans-nonachlor, pp'-DDE, mirex. The second solvent, 50 mL of 15:85 DCM:hexane, separated chlordanes, hexachlorocyclohexanes, cis-nonachlor, pp'-DDD and -pp'-DDT. The third solvent poured into the column, 130 mL of 60:40% DCM:hexane eluted heptachloroepoxide and dieldrin.

The analyses of PCBs and other organochlorines were completed by running 2 µL of the 1 mL of rotoevaporated sample in a Hewlett-Packard 5890 gas chromatograph equipped with a 5973 mass selective detector (MSD) and a 5973 autosampler with a DB-5 column (Chromatographic Specialties, Brockville, ON, Canada). The carrier gas in this machine was high purity helium and the mode of injection was splitless. The mass spectrum obtained after machine run was analysed using the MSDChem software based on their retention time and major ion. This was compared against a working standard diluted from a certified standard (Accustandard Quebec Ministry of Environment PCB Congener Mixture and Accustandard Custom Organochlorine Pesticide mixtures).

The accepted GLIER standard protocol was followed to determine analytical accuracy. This was done by determining the spiked surrogate standard's recovery. The samples were spiked with 100 µL of a surrogate standard (a mixture of C¹³ labelled PCB 37, PCB 52 and PCB153 in a concentration of 200 ng/mL during the sodium sulphate extraction period. A blank and a standard homogenate sample (in house Detroit River fish homogenate) were analysed together with each five sets of samples. Recovery rates of 65% to 135% for different samples were within an accepted range. The (mean ± SD) of PCB congeners was 52 (72.45 ± 24.85), 138 (276.76 ± 82.59), 153 (568.53 ± 185.61), 180 (472.97 ± 134.17) and 194 (85.45 ± 24.97) ng/g wet weight of in-house reference homogenate, which was within the acceptable range of the standard values presented in laboratory control chart (mean ± 3 standard deviation).

Data analysis

The data are represented as proportions of insects of aquatic or terrestrial origin in each species and tree swallows for 3 years. The tree swallow diet data were mostly extreme % values so in order to attain a spread for the data and to attain normality, it was arcsine square root transformed prior to analysis. One-way ANOVA was used to

determine if tree swallow diet differed among years 2002, 2003 and 2005. The same analysis was used to compare the Σ PCB and Σ DDT in the food samples of three species. This data was log transformed to attain normality. Pairwise comparisons among means were performed using the Fisher LSD (least square difference) method at $p < 0.05$. All analyses were conducted using Statistica[®] version 6.1 software (Statistica, 1997). All organochlorines and PCB congeners were expressed as ng/g wet weight (ww). Σ PCB concentration is the sum of the concentrations of the 38 congeners (IUPAC #: 18, 17, 31/28, 33, 52, 49, 44, 74, 70, 95, 101, 99, 87, 110, 82, 118, 105, 151, 149, 153/132, 138, 158, 128, 156, 187, 183, 177, 171, 180, 191, 170, 201, 195, 194, 205, 208, 206 and 209) analyzed and quantified in each diet sample. p,p'-DDT, p,p'-DDE and p,p'-DDD are added up to get Σ DDT.

A PCA was conducted to see if there were any definable differences in the accumulation pattern of persistent and readily cleared PCB congeners and other organochlorines. The PCB congeners and other organochlorines are pre-designated to three groups based on Clark *et al.*, (1987) and Drouillard *et al.*, (2001; 2007). This was based on their persistence depending on the number of open *meta-para* positions on their biphenyl ring and their half life in different organisms.

Rapidly cleared chemicals, (half lives of 10-60 d), intermediate cleared (>60-150 d); slowly cleared chemicals (half lives > 150 d) were defined as follows:

Rapidly Cleared: OCS, dieldrin, PCB 31/28, 52, 49, 44, 70, 95, 101, 87, 110, 1511, 149.

Intermediate: HCB, PCB 74,

Slowly Cleared: oxychlordane, mirex, DDE, PCB 99, 105, 118, 153/132, 138, 158, 128, 156, 171, 177, 178, 180, 170, 183, 187/182, 195, 194, 201, 205, 206, 208, 209.

Results

The insects in the diet were identified to order except for Diptera and Odonata, which were identified to family. Nineteen samples (pooled samples of boluses collected from different nestlings from one box) were collected from tree swallow nestlings in 2002. Depending on the number of nestlings in the nest and feeding trips made by the parents, there was considerable variation in the number of insects from sample to sample (Tables

1, 2 (show the variability of insect groups in 2 different pooled boluses from 2 different swallow nest boxes) and 3 shows the major insect groups in the diet of three species). Diet of tree swallow nestlings was composed of 72 ± 32 % aquatic insects by biomass (n=19 pooled boluses with 1098 insects)) in 2002 and 88 ± 29 (n= 10 bolus samples with 190 insects) in 2003 and $82 \pm 32\%$ (26 bolus samples with 258 insects) in 2005. The mean proportions were not significantly different among years (ANOVA, $F(2,57) = 1.23$, $p=0.3$) (Figure1). Only 7% of the total diet biomass of house wren nestlings was composed of aquatic insects (number of insects= 129). The purple martin diet consisted of 42% aquatic insects by biomass (number of insects= 111). Most of the bolus samples collected for house wrens composed of only one or two insects per bolus and only 2 purple martin boluses were collected and rest of the insects were collected from purple martin nests as individual insects.

The most prevalent groups of insects in tree swallow nestlings' diet were midges (Chironomidae; 79%), followed by mayflies (*Hexagenia*; 16%), horse flies (Tabanidae; 3%) and soldier flies (Stratiomyiidae; 1%) in 2002. In 2003 mayflies (43%), followed by midges (34%), robber flies (Asilidae; 9%) and ants (Hymenoptera; 7 %). In 2005, mayflies (73%) followed by soldier flies (9%), midges (8%) and horse flies (8%) were the most represented groups in the diet of the nestlings.

The most prevalent insects in the purple martin diet were beetles (Coleoptera; 25%). The next most common groups were dragonflies (skimmers, Libellulidae, Odonata; 22%) followed by moths (Lepidoptera; 15%) and *Hexagenia* mayflies (12%).

The house wren nestlings' diet was mainly composed of grasshoppers (Orthoptera; 59%) followed by spiders (Araneae; 14%) and adult moths (12%), caterpillars (6%) and *Hexagenia* mayflies (7%) (Table3).

There was no significant difference in mean % moisture content (ANOVA, $F(2,4)=3.71$, $p>0.12$) (house wren= 36.71 ± 3.91 (n=3), purple martin= 33.54 ± 3.56 (n=2) and tree swallow= 25.94 ± 1.53 (n=2)) or lipid content (ANOVA, $F(2,4)=1.39$, $p>0.35$) (house wren= 4.55 ± 0.34 (n=3), purple martin= 8.1 ± 3.56 (n=2) and tree swallow= $4.54 \pm$

Table 1. Insects identified from the boluses of tree swallow nestlings from one nest box on a day (nest box # 15, date of collection July 4, 2002, and the nestlings were 7 days old)

Order	Family	Habitat	No. of insects
Diptera	Chironomidae	Aquatic	71
	Dolichopodidae	Terrestrial	8
	Asilidae	Terrestrial	7
	muscoid flies	Terrestrial	18
Ephemeroptera	Ephemeridae	Aquatic	4
Hymenoptera		Terrestrial	2
Hemiptera		Terrestrial	2

Table 2. Insects identified from the boluses of tree swallow nestling from another nest box on the same day (nest box # 19, date of collection July 4, 2002, and the nestlings were 7 days old

Order	Family	Habitat	No. of insects
Diptera	Asilidae	Terrestrial	3
	Chironomidae	Aquatic	15
	Dolichopodidae	Terrestrial	1
	Phoridae	Terrestrial	2
	Stratiomyiidae	Semi Aquatic	2
	muscoid flies	Terrestrial	2
	Chloropidae	Terrestrial	2
Ephemeroptera	Ephemeridae (<i>Hexagenia</i>)	Aquatic	5
Hymenoptera		Terrestrial	4
Odonata	Coenagrionidae	Semi-Aquatic	1

Table 3. Diet collection date, year of collection, % of aquatic fraction in the diet and the number of insects collected and the major insect groups in the diet of three species

Species	Date of collection	Number of insects	% of Aquatic insects	Major insects groups	Percentage
Tree swallow	June 26 - July30,2002	1098	72.49± 31.86	Chironomidae	79
				<i>Hexagenia</i>	16
				Tabanidae	3
				Stratiomyiidae	1
	July 9-17,2003	190	87.87 ± 29.42	<i>Hexagenia</i>	43
				Chironomidae	34
				Asilidae	9
				Hymenoptera	7
				Dolichopodidae	2
				<i>Hexagenia</i>	73
				Stratiomyiidae	9
				Chironomidae	8
Purple martin	June25-July 14, 2005	258	82.35 ± 32.02	Tabanidae	8
				Coleoptera	25
				Lebellulidae	22
				Lepidoptera	15
	June29-July14,2004	108	44.99± 4.19	Orthoptera	12
				<i>Hexagenia</i>	15
				Coenagrionidae	5
				Muscidae	3
				Chironomidae	2
				Orthoptera	59
				Araneae	14
				Lepidoptera (adults)	12
House wren	July9-17, 2005	129	7.26	caterpillars	7
				<i>Hexagenia</i>	7

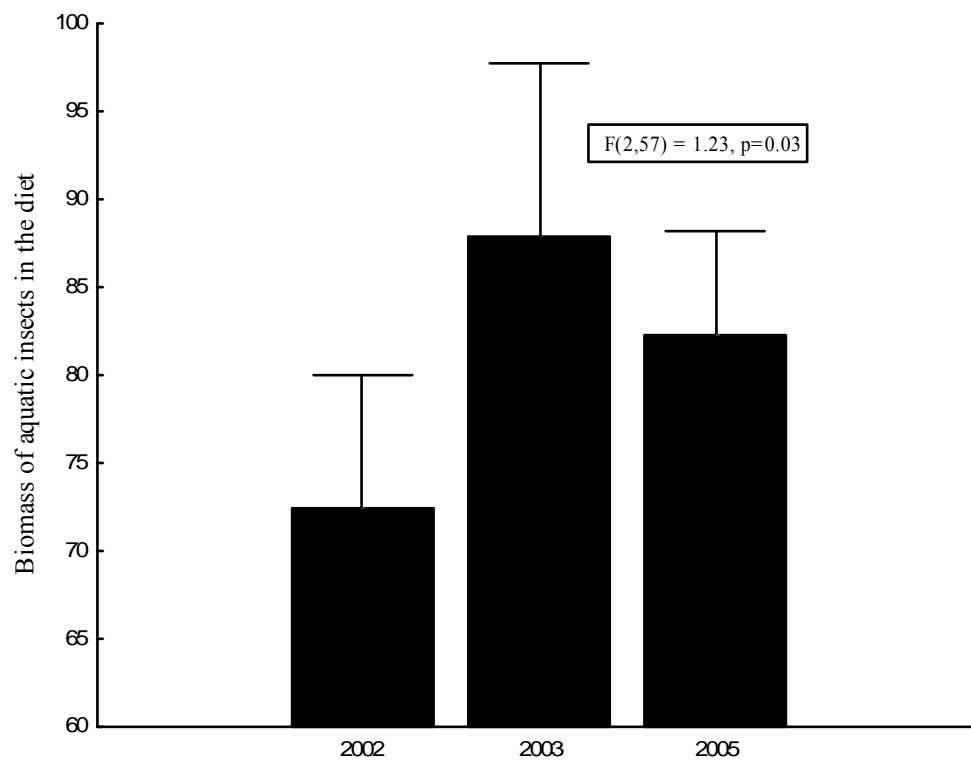


Figure 1. (Mean \pm SE) relative proportion (by biomass) of aquatic insects in the diet of tree swallow nestlings in three years (2002 n=19 pooled bolus samples; 2003 n=10 boluses and 2005 n=26 boluses)

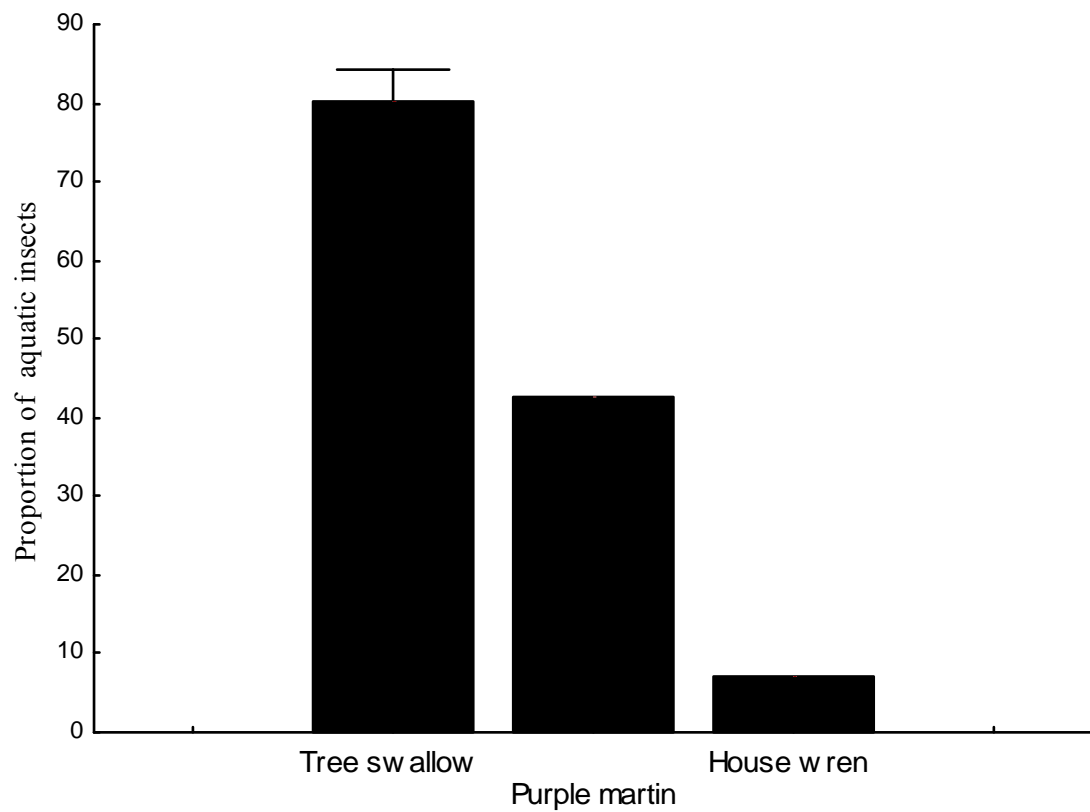


Figure 2. (Mean \pm SE) relative proportion (by biomass) of aquatic insects in the diet of tree swallow nestlings (n= 19 pooled samples + 36 boluses) and in purple martin nestlings (111 insects) and house wren nestlings (129 insects).

0.34 (n=2) among food samples of the three species. Σ PCB was significantly different in house wren food samples having the lowest value (ANOVA, $F(2,4)=9.68, p<0.03$). House wren food samples were different in Σ PCB concentration from other two species (house wren vs. purple martin $p<0.04$; Fisher LSD and between house wren and tree swallows $p<0.01$; Fisher LSD). There was no difference between purple martin and tree swallow dietary samples in Σ PCB concentration ($p>0.28$; Fisher LSD) (Figure 3 and Table 4). Σ DDT concentration of house wren food items was significantly higher (ANOVA, $F(2,4)=7.51, p<0.04$) than that found in the dietary insects of the other two species. House wren and purple martin diets were significantly different ($p<0.02$; Fisher LSD), whereas the tree swallow diet was not different from house wren diet samples ($p>0.07$; Fisher LSD) or from purple martin diet samples in Σ DDT content ($p>0.3$; Fisher LSD) (Figure 4 and Table 5).

Principle Component Analyses (PCA) showed that the first two factors explained 78% of the variation among variables (Figure 5 and Table 6). The variations in the axis were based on the distribution of organochlorines other than PCB congeners. All PCB congeners were positively associated with the first factor along with most of the organochlorines other than p,p' DDE, p,p' DDD and heptachlor epoxide. p,p' DDE, p,p' DDD were positively associated with factor 2 and heptachlor epoxide was negatively associated with factor 2. The variability displayed by wren and purple martin diets were mainly related to their organochlorine pesticide concentrations.

The mean % of readily cleared congeners was compared among the diets of three species. The house wren diet had a significantly lower percentage (18.33 ± 2.86) than purple martin (34.07 ± 1.11) and tree swallow (37.3 ± 4.09) diets. This difference was significant ($F(2,4)=17.42, p<0.01$). House wren diets were significantly different from tree swallows ($p<0.005$) and purple martins ($p<0.01$), but there was no significant difference between tree swallows and purple martins ($p>0.45$). The mean % of p,p' DDD over of p,p' DDE was not significantly different among diets of the three species ($F(2,4)=1.43, p>0.33$) (house wren = 10.48 ± 3.47 , purple martin = 6.7 ± 5.04 and tree swallow = 15.2 ± 2.46).

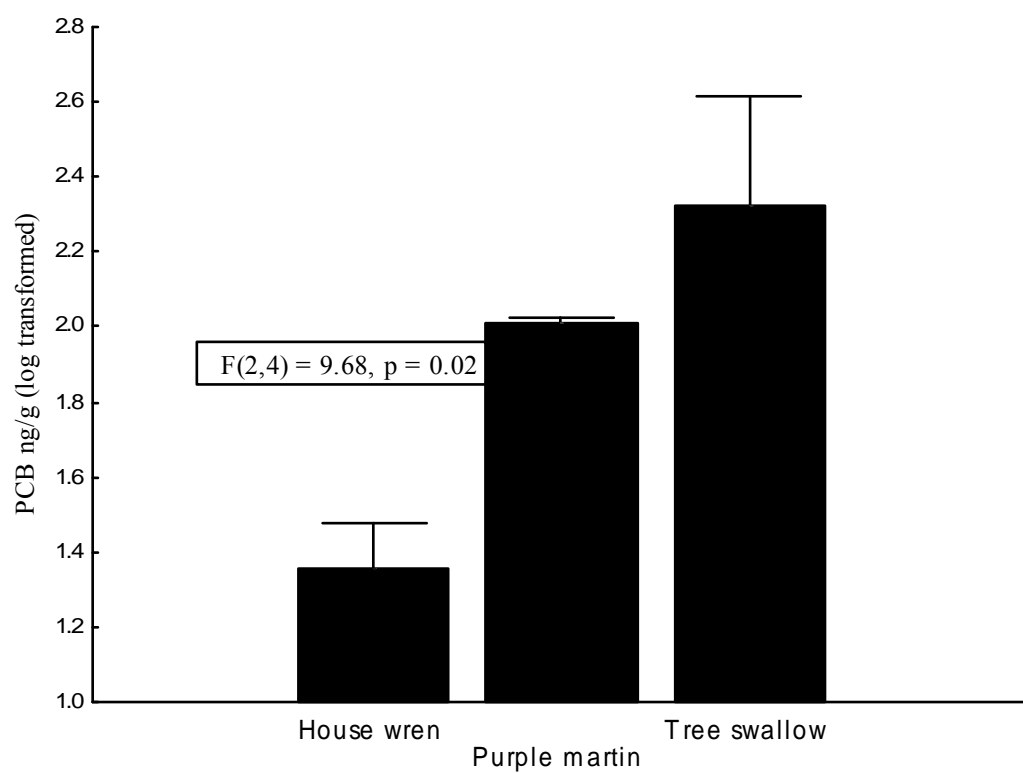


Figure 3. (Mean \pm SE) concentration of Σ PCB ng/g food insects of three species

Table 4. (Mean \pm SE) concentrations of PCB congeners (wet weight /g tissue) in the diets of three species

PCB congener	House wren	Purple martin	Tree swallow
PCB 18	0.26 \pm 0.31	0.45 \pm 0.13	3.68 \pm 3.28
PCB 17	0.29 \pm 0.25	0.16 \pm 0.07	1.03 \pm 0.52
PCB 31/28	0.28 \pm 0.14	1.05 \pm 0.02	7.9 \pm 6.85
PCB 33	0.14 \pm 0.07	0.34 \pm 0.17	3.33 \pm 2.76
PCB 52	0.37 \pm 0.21	2.61 \pm 0.05	7.85 \pm 5.56
PCB 49	0.29 \pm 0.16	1.54 \pm 0.08	3.75 \pm 2.09
PCB 44	0.21 \pm 0.1	1.28 \pm 0.19	4.27 \pm 2.67
PCB 74	0.17 \pm 0.03	1.13 \pm 0.25	2.72 \pm 1.77
PCB 70	0.28 \pm 0.14	1.53 \pm 0.02	4.72 \pm 3.38
PCB 95	0.41 \pm 0.23	3.64 \pm 0.01	11.1 \pm 6.86
PCB 101	0.67 \pm 0.26	5.83 \pm 0.12	13.9 \pm 8.45
PCB 99	0.78 \pm 0.35	3.24 \pm 0.25	6.35 \pm 3.07
PCB 87	0.32 \pm 0.18	2.5 \pm 0.48	6.28 \pm 3.75
PCB 110	0.66 \pm 0.27	5.93 \pm 0.08	14.52 \pm 8.96
PCB 82	ND	0.73 \pm 0.37	1.48 \pm 0.6
PCB 118	0.94 \pm 0.31	3.88 \pm 0.32	10.2 \pm 6.73
PCB 105	0.36 \pm 0.17	3.09 \pm 2.14	3.56 \pm 2.56
PCB 151	0.32 \pm 0.1	1.91 \pm 0.08	4.5 \pm 2.22
PCB 149	0.93 \pm 0.4	5.74 \pm 0.02	14.79 \pm 8.17
PCB 153/132	4.58 \pm 1.66	16.17 \pm 0.24	35.39 \pm 18.82
PCB 138	2.54 \pm 0.87	11.82 \pm 0.3	27.73 \pm 14.54

Table 4. (Mean \pm SE) concentrations of PCB congeners (wet weight /g tissue) in the diets of three species (cont'd.)

PCB congener	House wren	Purple martin	Tree swallow
PCB 158	0.17 \pm 0.78	0.61 \pm 0.24	2.22 \pm 1.28
PCB 128	0.41 \pm 0.23	1.91 \pm 0.02	4.23 \pm 1.83
PCB 156	0.32 \pm 0.15	0.82 \pm 0.12	2.06 \pm 1.26
PCB 187	1.52 \pm 0.6	3.04 \pm 0.33	7.29 \pm 3.85
PCB 183	0.45 \pm 0.25	1.71 \pm 0.07	4.84 \pm 2.44
PCB 177	0.47 \pm 0.11	1.5 \pm 0.36	3.66 \pm 1.2
PCB 171	ND	0.7 \pm 0.06	1.68 \pm 1.05
PCB 180	3.98 \pm 1.45	9.01 \pm 0.19	20.84 \pm 9.57
PCB 191	ND	ND	ND
PCB 170	1.38 \pm 0.51	4.5 \pm 0.19	10.13 \pm 5.25
PCB 201	0.84 \pm 0.43	1.03 \pm 0.2	3.37 \pm 1.76
PCB 195	0.12 \pm 0.06	0.46 \pm 0.1	1.65 \pm 0.85
PCB 194	1.19 \pm 0.39	2.3 \pm 0.01	6.31 \pm 3.32
PCB 205	ND	ND	ND
PCB 208	0.23 \pm 0.11	0.18 \pm 0.09	0.3 \pm 0.15
PCB 206	0.34 \pm 0.17	0.45 \pm 0.23	1.7 \pm 0.67

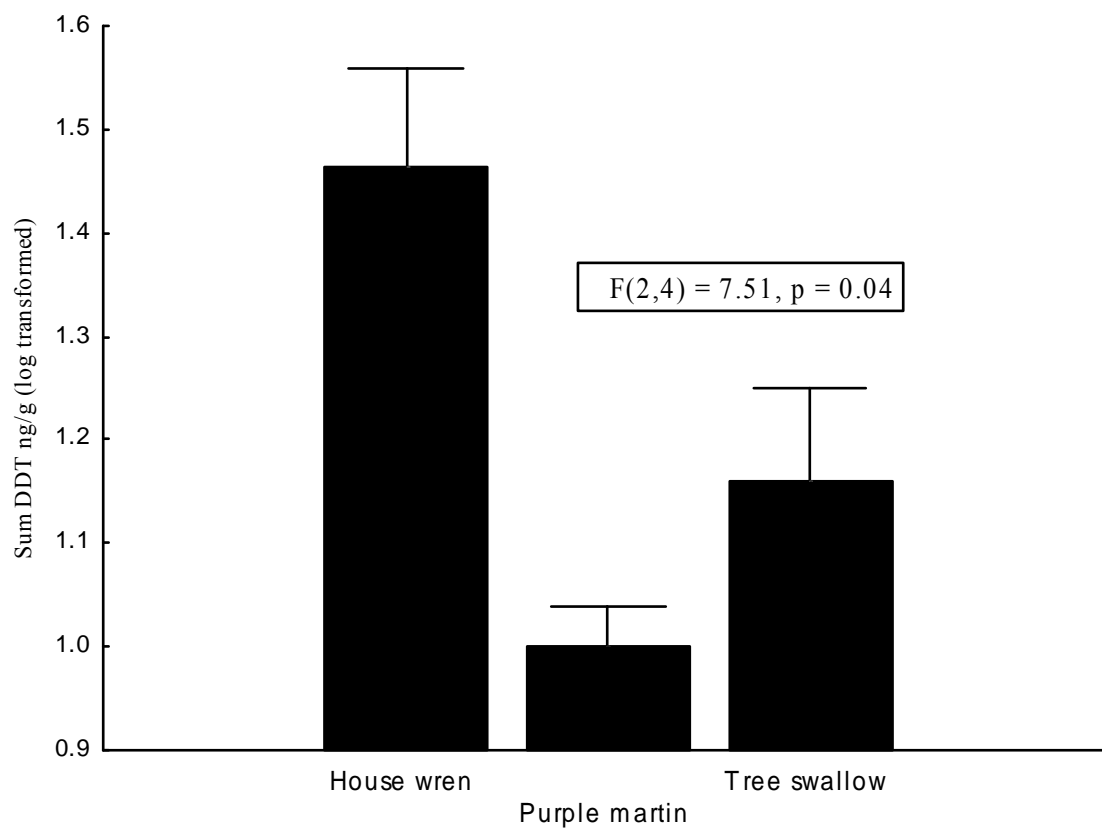


Figure 4. (Mean \pm SE) concentration of Σ DDT ng/g food insects of three species

Table 5. (Mean±SE) concentration of organochlorine pesticides (ng/g wet weight) in the diets of three species

	House wren	Purple martin	Tree swallow
Compound			
1245-TCB	ND	ND	ND
1234-TCB	ND	ND	ND
QCB	0.09	0.2±0.02	0.21±0.04
HCB	0.26±0.08	0.39±0.01	0.98±0.12
a-BHC	ND	0.4±0.2	0.41±0.09
b-BHC	0.98±0.82	1.28±0.61	3.3±0.41
g-BHC	9.23±7.34	4.45±3.03	7.57±3.79
OCS	0.3	ND	0.64±0.18
Heptachlor Epoxide	ND	0.78±0.32	ND
Oxychlordane	ND	ND	ND
trans-chlordane	ND	ND	ND
cis-chlordane	ND	0.38	0.36±0.18
trans nonachlor	0.2±0.05	0.38±0.14	0.58±0.01
p,p'-DDE	20.73±12.69	8.43±0.26	11.78±2.23
Dieldrin	ND	6.77±3.39	3.82±0.68
p,p'-DDD	2.54±2.28	0.58±0.44	1.84±0.63
cis-nonachlor	3.53±2.12	0.31±0.01	0.28±0.1
p,p'-DDT	7.29±5.29	1.03±0.19	1.19±0.18
Mirex	0.11±0.22	0.74±0.37	0.88±0.21

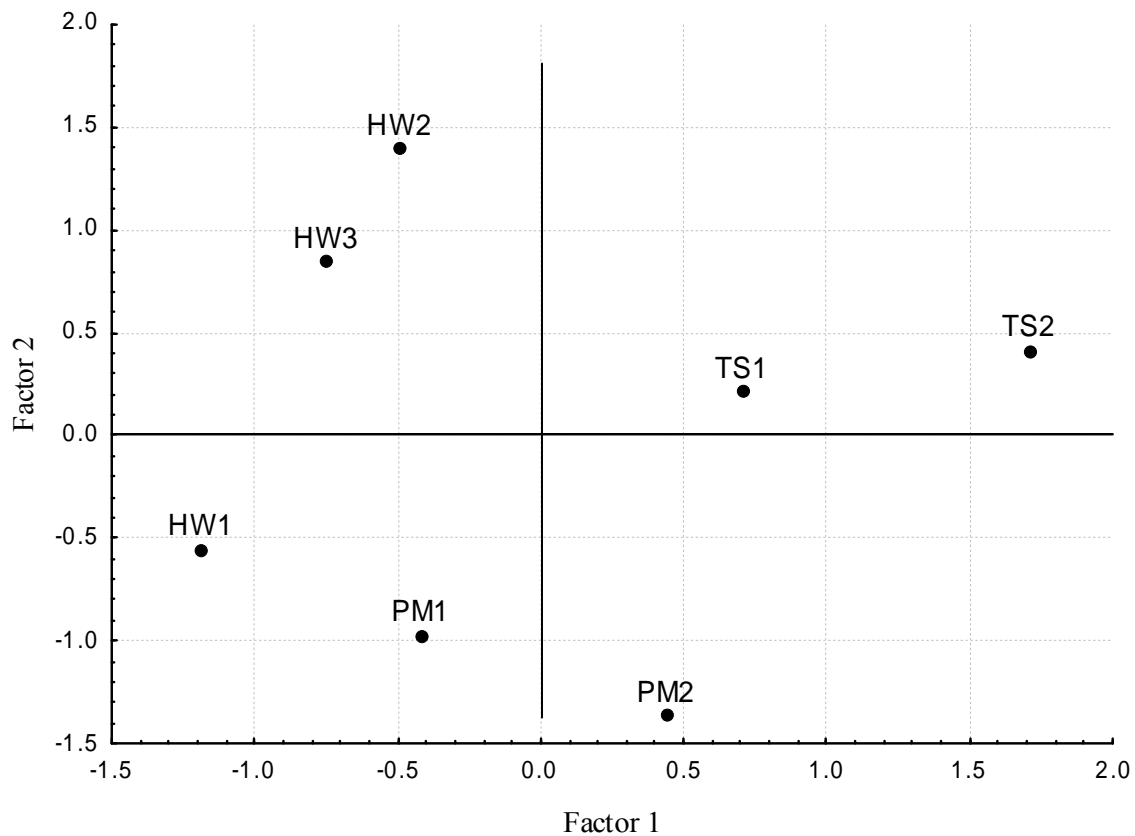


Figure 5. Plot of principal components 1 and 2 based on the contribution of different PCB congeners and other organochlorines in the food insects of three species (HW1-3= house wren food, PM 1 and 2 = purple martin food and TS1 and 2= tree swallow food)

Table 6. Factor loadings of the food insects of three species (Extraction: Principal components)

Variable	Factor 1	Factor 2	Variable	Factor 1	Factor 2
QCB	0.84	-0.34	PCB 99	0.99	-0.03
HCB	0.94	0.18	PCB 87	0.94	-0.02
a-BHC	0.81	-0.23	PCB 110	0.98	-0.18
b-BHC	0.81	0.22	PCB 82	0.78	0.03
g-BHC	-0.11	0.15	PCB 118	0.97	0.00
OCS	0.65	0.45	PCB 105	0.86	-0.05
Heptachlor	0.09	-0.78	PCB 151	0.98	-0.13
Epoxide					
cis-chlordane	0.73	-0.33	PCB 149	0.99	-0.10
trans	0.87	-0.25	PCB	0.98	0.04
nonachlor			153/132		
p,p'-DDE	-0.06	0.87	PCB 138	0.99	-0.03
dieldrin	0.49	-0.18	PCB 158	0.94	0.19
p,p'-DDD	0.09	0.85	PCB 128	0.91	0.07
cis-nonachlor	-0.59	0.72	PCB 156	0.93	-0.08
p,p'-DDT	-0.67	0.06	PCB 187	0.87	0.29
mirex	0.76	0.35	PCB 183	0.99	0.03
PCB 18	0.74	-0.15	PCB 177	0.92	0.05
PCB 17	0.16	-0.26	PCB 171	0.93	-0.34
PCB 31/28	0.85	-0.16	PCB 180	0.91	0.27
PCB 33	0.83	0.30	PCB 170	0.97	0.10
PCB 52	0.94	0.01	PCB 201	0.74	0.61
PCB 49	0.93	0.04	PCB 195	0.96	-0.06
PCB 44	0.94	-0.15	PCB 194	0.85	0.32
PCB 74	0.95	-0.19	PCB 208	0.54	0.21
PCB 70	0.94	0.04	PCB 206	0.57	0.65
PCB 95	0.99	-0.11	Expl.Var	34.19	5.06
PCB 101	0.98	-0.17	Prp.Totl	0.68	0.10

Discussion

The nestlings of all three species were provided with a diverse mix of insects. The composition of insects used by each species reflected their parents' foraging methods and locations. Insects were collected by aerial hawking or by gleaning in the bushes. Species differed in their foraging locations, including capture above water bodies vs. above terrestrial habitat, and low vs. high in the air column. The swallow nestlings' diets were studied from June 26th to July 30th in 2002 and from July 9th to 17th in 2003 and from June 25th to July 14th in 2005. There was not much difference in composition in the distribution of the major groups of insects in their diet. The two major groups were midges and mayflies. The emergence of mayflies was between June 12th to August 26th in 2002, with a mass emergence at the end of June. The mayfly emergence period in 2003 was from June 10th to August 10th (Sharma, 2004). This is consistent with previous observations that swallows are opportunistic feeders (McCarthy 2001; Quinney and Ankney 1985).

In our study of 2005 we missed the peak emergence and collected dietary samples late in the season, which is why there was slightly less biomass of mayflies in their diet. Previous studies have indicated that swallows prefer aquatic insects, and in most cases more than 80% of dietary items were aquatic origin (Smits *et al.*, 2000; Mengelkoch, 2004). We confirmed that the preponderance of tree swallow diet is composed of aquatic insects with $\geq 80\%$ being from aquatic habitats. Mayfly (*Hexagenia*) larvae live in burrows in lake-bottom sediments. Their larval stage typically lasts 1-2 years, but the terrestrial adults live a maximum of 3 days, and don't feed during that time. This is true for midges too. Their larval stages in the aquatic sediments are very long compared to their life after their eclosion. Traditionally it was believed that Chironomidae are short lived and non feeding but adults of some species can live from few days to few weeks if they are able to feed on honey dew and nectar (Oliver, 1981). Nevertheless, their aquatic life cycle phase is much longer than their terrestrial life term. Consequently, tree swallows are suitable candidates for the study of bioaccumulation of aquatic sediment bound pollutants.

We collected diet insects of purple martin nestlings from June 29th to July 14th in 2004. Even though the diet collection period for purple martin nestlings and swallow

nestlings overlapped, there was a significant difference in dietary fractions of aquatic and terrestrial insect composition and very noticeably different in the contributions of major insect groups. The most commonly represented diet items were beetles and dragonflies. Dragonflies are good fliers and often fly high up in the air as do purple martins. Thus, their spatial niches overlap to a great extent. Dragonflies have an aquatic larval period but they also have extended adult terrestrial phase. As these insects have both aquatic and terrestrial feeding regime in their life cycle they are capable of transferring both terrestrial and aquatic pollutants. The other two major components in purple martins' diet, beetles and moths, are both terrestrial. Mayflies were also represented in purple martins diet but only to a minor extent. Some of the major insect groups in our study (Libellulidae, Coenagrionidae, Musidae and Chironomidae) were also found to be important diet items by Walsh (1978) for two consecutive years.

House wren diets were collected from July 9-17, 2005. The most abundant groups represented in wren diet were all terrestrial insects. Beal (1987) and Beal *et al.*, (1916) as reported by Gross (1948) found that more than 90% of wren diets were composed of terrestrial insects. We found the same composition in our study. Skutch (1953) stated that the most important food items in wrens' diet were small grasshoppers, spiders, cockroaches and hairless caterpillars. In this study too three most important groups were grasshoppers spiders and caterpillars. Beal (1987) and Beal *et al.*, (1916) as reported by Gross (1948) also found grasshoppers, moths and their caterpillars, and spiders to be major components in their diets. Judd (1900) (reported in Gross 1948) found besides the above mentioned groups and mayflies as a major component. In Judd's study of a day of observations from 9.35 a.m. to 12.40 p.m. the food included 4 spiders, 5 grasshoppers, 17 mayflies and 20 caterpillars. This composition together with our results shows the consistency in their spatial feeding niche and their preference for certain food items. Whereas tree swallow parents and purple martins give a mixture of insects as a bolus to their young ones, the wren bolus consisted of a single prey item most of the time.

The contaminant contents in the diet of different species were reflective of their feeding strategy. The specific niche from which they fed showed the difference in accumulation. The differences in their diet compositions were reflected in the contaminant loads of Σ PCB and Σ DDT received by the nestlings from their diets.

Environmental contaminants are accumulated by insects from their habitats and they transfer it to their consumers. The process of larval development and the habitats in which they develop influence the types of contaminants that they may accumulate and pass on to their predators. All three species had the expected proportionate mixture of persistent, readily cleared congeners. The difference in the accumulation of p,p' DDE to p,p'DDD showed they were mirroring the soil conditions of Point Pelee National Park, in which DDT was used until it was banned in the 1970s.

It is very important to know the foraging behaviour and diet of animals to ascertain their relative position in their habitat (trophic niche) and to their ecology as a whole. The three species live in the same spatial niche in a common habitat and share many characteristics. However, they exploit different niches for their feeding so there is niche partitioning as far as their feeding behaviour is considered. The species maximize their ability to provide food for their nestlings by segregating to different levels to exploit available food resources from the same area. During the nesting stage, the diet affects birds' rate of growth, their size, their health, time required for fledging, fledging rate and their survival. The post hatching success of the offspring also depends on the parents' ability to supply adequate food during their nestling period (Horne and Bader, 1990). In migratory birds, the quality and quantity of food received is a critical element in the preparation for the migration to overwintering locations. Animals' diets are an important determinant in the bioaccumulation of different toxic chemicals. So it is very important to study the diets of animals, especially higher trophic level organisms, before they are used for bioaccumulation/bioindicator studies.

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Chapter 4: Bioenergetics-based models for the accumulation of PCB and DDT by nestlings of three passerine species - tree swallow (*Tachycineta bicolor*), purple martin (*Progne subis*) and house wren (*Troglodytes aedon*) at Point Pelee National Park, Ontario, Canada

Introduction

Migrant insectivorous passerine birds, nestlings and eggs are increasingly used for biomonitoring the accumulation of persistent organic pollutants and to assess their biological effects (Dauwe et al., 2006; 2009; Spears *et al.*, 2008; Jayaraman *et al.*, 2009). They are used as sentinels for local pollution because their home ranges are small especially when they raise their nestlings. This study is based on three insectivorous passerine species - tree swallows (*Tachycineta bicolor*), purple martins (*Progne subis*) and house wrens (*Troglodytes aedon*). Tree swallow nestlings have frequently been used as sentinel species of organic contaminant accumulation, and especially of local aquatic sediment-bound pollution (Bishop *et al.*, 1995, 1999, Custer *et al.*, 1998, 2000, Froese *et al.*, 1998, Secord *et al.*, 1999, Smits *et al.*, 2000; 2005; Brasso and Cristol, 2008; Jayaraman *et al.*, 2009). They are suitable model organisms for many reasons, including their short nestling period, hardiness and resilience (McCarthy and Secord, 1999) and their ready availability and tractability (Jones, 2003). Because they readily accept nest boxes, one can establish colonies in desired locations (Bishop, *et al.*, 1995, 1999; Roof and Harris, 2000; Echols *et al.*, 2004). Tree swallow parents feed their nestlings with locally available, flying insects of both aquatic and terrestrial origin (McCarthy and Winkler, 1999). Aquatic insects dominate their diet, and in most cases over 80% of the biomass ingested is of aquatic origin (Mengelkoch, 2004; Chapter 2).

Purple martins share all the above qualities. However, unlike tree swallows, which forage largely over water, purple martins tend to feed over land and exploit a broad range of terrestrial insects (Sibley, 2001). Consequently, purple martins are more likely to biomagnify the pollutants associated with terrestrial soils than tree swallows.

A third passerine species of importance that shares the same breeding habitats as tree swallows is house wrens. Wrens are insectivorous but they adopt a different mode of feeding - gleaning, i.e., they collect insects from the leaves. Although their food is similar to purple martins in being terrestrially-based, it has a different composition than the aerial

feeder. Purple martins and house wrens are used for monitoring terrestrial food webs. The feeding strategy and foraging methods of organisms are very important components to be considered when studies are conducted on bioaccumulations of pollutants (Sagerup *et al.*, 2002).

All three species mentioned above breed in and near Point Pelee National Park, Ontario. This park is an extremely important area for spring and fall migrations of birds (Fletcher, 1997). It lies in an area where the Atlantic and Mississippi flyways overlap. The two flyways are very prominent continental routes along which many birds pass during migrations (Proctor and Lynch, 1993). There was heavy use of DDT in the park by farmers during 1950s and 1960s to control agricultural pests and biting insects. In the 1970s, DDT use was banned from the park, but the levels of DDT in soil in the park environment remain higher than guidelines imposed by the government. The contaminated areas are two former agricultural areas and two former areas intensively used by visitors (Natural Heritage Soil Contamination Study, 2001; Smits *et al.*, 2005). The sediments of the adjacent marshes and nearshore Lake Erie around the park have significant amounts of DDT and PCBs (Parks Canada, 2003).

The three species selected for the study have different hatching and fledging biomasses and the time duration to acquire fledging biomasses are different. House wrens require the fewest days to fledge, and fledglings have relatively low biomass. Purple martins develop the most slowly and have the greatest biomass at fledging. Tree swallow are intermediate in development time and biomass at fledging (Brown, 1997; Johnson, 1998; Robertson *et al.*, 1992).

Growth, best described as an increase or change in size and weight of an organism in days or with age, is the sum total of many physiological processes (Ricklefs, 1968; 1969; 1973). The proportions of different organs of the body and the shape and relative sizes of different organs change in a manner specific to particular organisms, but all follow a pattern. A logistic equation is the best fitting equation to describe the growth curve of all birds (Ricklefs, 1967; 1968). The growth curves can be described and

compared by three different parameters namely magnitude, form and rate (Ricklefs, 1968).

The overall increase in growth during the nestling period must approach adult weight, and this is described as a standard curve. However, in some cases the accumulated biomass will exceed adult weight and then decline to adult weight. In certain other cases it will remain at a level below adult weight and then gradually increase to adult weight. These differences in growth pattern are described as the magnitudes of the curve.

The form is mainly sigmoidal, i.e. the slope of the growth curve (the absolute rate of growth) increases to an inflection point and decreases thereafter. The point of inflection is a very important measure used to compare growth form variation. The inflection point may be reached at different periods in different bird species, and after nestlings have attained different percentages of their adult body weight. The speed at which the adult weight is attained is the most important feature of growth in birds.

These differences in their growth will affect their food needs (consumption rate), and consequently their total contaminant accumulation rate. Even though the nestlings share the same spatial niche their trophic niches differ, and this will also affect their contaminant accumulation pattern. The accumulation rate and pattern will also be influenced by diet insects' habitat and trophic position in their respective habitats (terrestrial and aquatic). The postulate for this study is that the accumulation of Σ DDTs and PCBs will differ in quantity and proportion in the nestlings of three species, reflecting their growth and consumption rates, and the composition of their diet. There will be differences in accumulation in different species based on how much they receive from their mother through the eggs and what they accumulate from their developing ground.

The objectives of this study are to

1. investigate the biomagnification of organochlorine pollutants based on bioenergetics principles and the type of food they consume for three avian species breeding in the vicinity of Point Pelee National Park

- a. estimate the contribution from egg and on site accumulation of pollutants from food by each species
2. compare the observed accumulation of Σ DDT and Σ PCB congeners in the diet, eggs and nestlings of the three species
3. evaluate biotransformation capacity of nestlings across species

In order to apply the bioenergetics based model for three species, their growth for each day was measured and developed growth curves, and estimated growth rate for each species. This was useful to compare the growth rates across three species. The consumption rate and total consumption were estimated for each species based on growth rate and final body weight of nestlings.

Materials and methods

In March 2002, we erected 50 standard nest boxes for tree swallows (Appendix 1,2,3 and 4). We determined the growth of nestlings, collected bolus samples from the birds to see what they are eating (Chapter 2). We relocated 21 of these nest boxes in the summer of 2005 to another location in the park to do some feeding experiments, and swallows were studied in those boxes again (Appendix 5).

House wrens were studied in 2005 in the same 21 boxes. The tree swallow nestlings in all boxes had been lost to predation in 2005, and the nest boxes became occupied by wrens later in the same summer. Growth measurements were made and eggs, food samples and nestling tissues were collected for contaminant analysis.

Purple martin nestlings were studied in nest boxes in Erie Shores Golf Course just outside the park. Growth measurements, food samples and tissues were collected in summer months of 2004.

Diet sample collections

Bolus samples were collected from the parents before feeding the nestlings to determine the diet composition (Chapter 3). For collecting diet samples from tree swallow nestlings and wren nestlings we used the ligature method. But for purple martins

we used the parent trapping method, a method to obtain high quality boluses by trapping adults in the nest boxes when they visit the nest box to deliver food to the nestlings. We trapped the adults when they entered into the nest and retrieved the food boluses before it was delivered to the nestlings. We devised a trap door that could be operated from a distance to trap an adult immediately after it entered the nest boxes with the food. Once trapped in the nest, the adult sometimes delivered the food to the nestlings or swallowed the bolus themselves. At other times they could drop the insect bolus in the nest cup or surroundings into the nest materials. Another difficulty was that many insects were alive and escaped from the bird's beak during the process of collection. We could collect only very limited a number of insects by this method. Consequently, we pooled these specimens with aerial sweep samples of the same taxa with which to do contaminant analysis.

For the ligature method, the nestlings were collared when they were at about the mid age of their time to fledging. An elastic band or collar was placed around the base of the throat of each nestling. This prevented them from swallowing food. We ensured that the rubber band collar was neither too tight nor loose. After fixing the collar in place, the nestlings were placed back into their nest cup. The feeding activities of the parents were observed from a distance. After around 45 min. or after the parents had made about five to six feeding trips we collected the bolus samples from individual nestlings using a curved hemostat (Smits *et al.*, 2005; Chapter 3; Appendix 2).

Insect specimens of the same taxa that made up the diet of each passerine species were collected in large quantities for contaminant analysis. Brush sweep samples were taken to collect insect samples for wrens, and aerial sweep samples were made for purple martin food items. For tree swallows we analysed two samples that consisted of the pooled boluses collected from the nestlings by ligature method.

Tissue collection

We randomly collected nestlings of each species at around their fledging age for contaminant analysis. Five wren nestlings, 4 swallow nestlings and 4 purple martin nestlings were collected in the years during which they were studied. The nestlings were anaesthetized using inhalant anaesthetic (Halothane[®]; Halocarbon Laboratories, River

Edge, NJ, USA), immediately after collection and euthanized by exsanguinations. Their feathers, internal organs and breast muscles were removed and kept for other studies. The rest of the tissues were stored frozen at -20°C until chemical analyses were performed.

Egg collection

We collected 4 tree swallow eggs and 8 house wren eggs in 2005 and 5 purple martin eggs in 2004 (Chapter 2). Two eggs of house wrens were pooled to make a sample as the weight of one egg was not enough for chemical analysis. The eggs were also immediately frozen and stored at -20°C until chemical analysis.

Sample preparation and chemical analysis

Eggs (yolk and albumen), homogenized nestling tissues, and dietary invertebrates were used for contaminant analysis. Each sample was dried by mixing with 35 g of sodium sulphate (VWR, Mississauga, ON) in previously cleaned and hexane rinsed mortars and pestles. Samples were then transferred to glass columns for solid-liquid extraction with 50 mL of dichloromethane (DCM): hexane (1:1) (Fisher Scientific, Ottawa, ON). Each column was spiked with a surrogate standard (a mixture of C^{13} labelled PCB 37, 52 and 153 of concentration 200ng/mL) to determine the recovery rate of the chemicals in the sample. An additional quantity of 250 mL of the same mixture was added to each column and allowed to stand for one h. After one h, each column was eluted, the extracts were rotoevaporated, and 10 percent of each sample was used for lipid determination by drying the samples in tin foil plates in an oven and determining the weight of remaining lipids. We followed the method devised by Drouillard *et al.*, (2004) to determine the lipid content.

Because of high lipid content in the eggs and some other samples, additional cleaning for removing excess lipids of those samples were done using gel permeation chromatography (GPC). The GPC column was filled with porous beads (SX-3 biobeads, BioRad, Mississauga, ON) and organic solvents. It was possible to separate lipids from organochlorines with this procedure as lipids move faster than the contaminants in this column. The solvent used was 50:50 DCM:hexane. All samples were cleaned and separated further by florisil chromatography. The column solid florisil (8g of 60-100

mesh florisisil, VWR, Mississauga, ON) was soaked in hexane. Three different solvents (different ratios of DCM and hexane) were poured through the florisisil column, which separated different fractions of the organochlorines and each fraction was collected separately. The first solvent poured was 50 mL of hexane, which separated PCBs (including mono-ortho substituted congeners), chlorobenzenes, octachlorostyrene, trans-nonachlor, pp'-DDE, mirex and photomirex. The second solvent was 50 mL of 15% DCM:hexane, which separated non-ortho substituted PCBs (77, 126, and 169), chlordanes, hexachlorocyclohexanes, cis-nonachlor, pp'-DDD and -pp'-DDT. The third solvent was a 130 mL aliquot of 60% DCM:hexane, which separated heptachlor epoxide and dieldrin.

Analysis were completed by using a Hewlett-Packard 5890 gas chromatograph fitted with a 5973 mass selective detector (MSD) and a 5973 autosampler with a DB -5 column (Chromatographic Specialties, Brockville, ON). The injections were made in splitless mode and the carrier gas used was very pure helium. The volume used for injection was 2 μ L. The oven temperature was programmed at different levels with the lowest being 90⁰C and the highest being 280⁰C. The chromatogram was analysed using the MSDCHEM software. Different PCB congeners and other organochlorines were identified and quantified using their retention time and major ion against working standards. This working standard was prepared by diluting certified standards (Accustandard Quebec Ministry of Environment PCB Congener Mixture and Accustandard Custom Organochlorine Pesticide mixtures).

Accuracy of analyses was by quantifying the spiked surrogate standard's recovery. Blank and standard homogenate (in house Detroit River fish homogenate) analyses were performed with each five sets of samples. Recovery rates for all samples fell within a standard range (65% to 135%). The concentration ng/g wet weight (mean \pm SD) of PCB congeners 52 (89.08 \pm 13.2), 110 (181.7 \pm 30.34), 153 (265.19 \pm 56.64) and 138 (298. 22 \pm 64.34) of in house reference homogenate were within the range of the laboratory control chart values (mean \pm 3 standard deviation).

Bioenergetics-based model

Bioenergetics is the application of thermodynamic principles to biological processes. A bioenergetics model is a mathematical accounting of the bioenergetics and is intended to predict the bioaccumulation of pollutants in an organism by quantifying the fundamental biological processes of that organism (deBruyn and Gobas, 2006). The bioenergetics model is based on a balanced energetics equation (Winberg's equation) in which, for non-reproductive organisms, all energy consumed (C) is allocated between metabolism (R), egestion (F), excretion (U), and growth (G)

$$C = R + F + U + G \quad (\text{Cianelli } et al., 1998).$$

(All units are expressed in kcal/g/d).

Growth (G) can be separated into somatic (**G_{gro}**) growth and gonadal /reproductive growth (**G_{rep}**)

Metabolism (R) can be distinguished as **basal metabolism (BM)**, **thermoregulatory metabolism (TM)**, **activity costs (AM)** and cost for digestion of food, **specific dynamic action (SDA)**.

Egestion (F) is the sum total of the unassimilated energy egested as feces and

Excretion (U) is the assimilated energy later excreted as urea and ammonia.

From the above expansions it is evident that the consumed energy can be allocated to or partitioned into eight alternate fates:

$$C = G_{gro} + G_{rep} + BM + TM + AM + SDA + F + U$$

When it is rearranged for growth the equation becomes:

$$G = C - (BM + TM + AM + SDA + F + U)$$

$G_{tot} = G_{gro} + G_{rep} = BW$ (body weight) growth attained /d can be measured as body weight change/d (body weight can be converted to kcal/g/d by multiplying it with caloric density of nestling body tissue at different ages)

All the energy used by an organism excluding the excretory waste is termed as total metabolizable energy (TME). Growth or Production (P) for birds are estimated as a fraction of TME, so growth efficiency or energy allocation during growth can be calculated as P/TME (Williams and Prints, 1986).

All the energy requirements of an organism are based on the growth stage of that organism and so are directly related to its BW, since BW is an indication both of cumulative growth and of an organism's developmental stage (Naggy, 1987; Blem, 1973; Diehl and Mircha, 1973; Weathers and Sullivan, 1991; Weathers, 1992; Ricklefs *et al.*, 1996).

All biological processes require energy, which is provided through their food. So energy expenditure and growth of an organism can be directly coupled to its food consumption. Thus, the energy requirements or the Gross Energy Intake (GEI) or the consumption of an organism can be predicted from an allometric scaling equation based on its body weight (Lasiewski and Dawson, 1967; Kendeigh, 1970; Vleck and Vleck, 1979; Naggy, 1987; Naggy *et al.*, 1999; West *et al.*, 1997; Gremillet *et al.*, 2003).

If the gross energy intake or realized consumption by the nestling is estimated, the rate of accumulation of organochlorine pollutants by nestlings of birds (ΔBB , in $\mu g \text{ bird}^{-1} \text{d}^{-1}$) can be calculated by the following equation

$\Delta BB = (C \cdot BW \cdot [Pc] \cdot ASSIM) / CD_1$ where ΔBB = accumulation rate ($ng \text{ bird}^{-1} \text{d}^{-1}$), Pc = concentration of pollutant in the diet insects ($ng \text{ g}^{-1}$ insect tissue wet weight), $ASSIM$ = proportion of assimilation of pollutant from the diet, CD_1 = caloric density of insects ($kcal \text{ g}^{-1}$ wet weight) (Nichols *et al.*, 1995; 2004)

Total contaminant in nestling = maternal contribution (egg) + pollutant accumulated from diet.

Model parameterization

The model parameters used are derived from the bioenergetic literature, mainly from Blem (1973; 1975), Weathers and Sullivan (1991) and Nichols *et al.*, (1995; 2004). We estimated consumption of nestlings of the three study species from the absolute growth measurements we made in the field. Absolute growth is defined as the increase in biomass of an animal over a period of time $(W_{t_2} - W_{t_1}) / (t_2 - t_1)$. We weighed the nestlings daily until they fledged, which provided a measure of absolute growth per day for each species. However, the energy content of nestling tissues changes during growth due to accumulation and storage of fat and changes in water content and other physiological changes (measured as energy density of tissues). Thus, the amount of energy accumulated must also be accounted for when we calculate the absolute production of tissues per day. Equations for calculating caloric density (CD) were developed by Blem (1993; 1995) and Holmes *et al.* (1997) between the weight of nestlings and energy content of their tissues. We adopted the value from the relationships provided by Blem (1995) and Nichols *et al.* (1995; 2004).

$$CD_B (\text{Caloric density of bird}) = BW * 0.037 + 0.477$$

Multiplying the CD of bird tissues at different ages by their weight gain at those ages gave the total production per day per bird.

We calculated the total consumption by using values provided by Nichols *et al.* (2004) based on body weight.

$$C = 0.49BW^{0.23} \qquad \text{Nichols } et al. (2004)$$

We adapted the equation provided by Nichols *et al.*, (2004) because they took into consideration all the values for energy requirements available in literature, for passerines

of same body mass range as our study species, for developing their equation. To the total R or ME (which includes all energy requirements such as **BM + TM +AM + SDA**) was added P. Combined fecal egestion (F) and urinary excretion (U) were added as a proportion (0.30) of ingested energy.

Nichols *et al.* 1994; 2004) adopted total FU as 30% of total consumption. This gave a net energy assimilation efficiency of 70%. This was in conformity with various other studies. Many studies have determined assimilation efficiency of passerine adults and nestlings. Assimilation efficiency for Cape Vultures was calculated by the equation

$$AE = \frac{GEI - (F+U)}{GEI} \times 100 \quad (\text{Komen, 1993})$$

and was found to range (for scavenger feeders) from 82% to 92% with a mean of $86.6 \pm 2.3\%$ for the entire growth period (Komen, 1993). The average assimilation efficiency recorded by Blem (1973) for six species of passerines ranging in weight from 6 g to 69.7 g is 75.3 percent. It was suggested that adults have higher AE than nestlings so we looked for values for nestlings. The assimilation efficiency for red-backed shrike nestlings averaged 70% (Diehl and Myrcha, 1973). Bryant and Bryant (1986) studied insectivorous altricial nestlings of two species of Pacific swallows, *Hirundo tahitica* and blue-throated bee-eaters *Merops viridis*, and found that the first species had an efficiency of 68% and other species around 57%. They also reported in the same study an average of assimilation efficiencies of different insectivorous passerines from different studies and from their two species as 71.3%. Tianen (1983) estimated the assimilation efficiency of nestling willow warblers at 67%. So the assigned 70% for energy assimilation efficiency value and an FU of 30% by Nichols *et al.* (1995; 2004) was in agreement with other studies.

The average consumption for tree swallows from our calculations and from the study of Diehl and Mircha (1973) for same sized red-backed shrikes nestlings were the same. The daily consumption of 28.0 kJ/d by wren nestlings on days 11 to 14 estimated by Dykstra and Karasov (1993) is not very much different from what we found in the estimate for consumption of wren nestlings of same age.

Most birds can maintain a constant and minimum expenditure of energy to maintain their body temperature in a specific environmental temperature range, which is

known as their thermoneutral zone (TNZ) within an upper and lower critical level. For a small bird like a zebra finch with a body weight of 10 g, the TNZ is between 32 and 40°C (Rijnberk and de Vries, 1995). In altricial birds the lack of homeothermy in nestlings is normally taken care of by the parents building insulated nests, having a large number of nestlings in each clutch, and by the parents' brooding behaviour. This normally keeps the TNZ within a range of 34-36°C. Furthermore, altricial birds are capable of developing some form of insulation at very young age (Ricklefs, 1974). Weathers and Sullivan (1991) suggested that a 36-37°C range within TNZ for nestlings of yellow-eyed juncos. For white leghorn chicks of age between 6 to 15 days an ambient temperature of 21- 32°C was the temperature for best growth (Ricklefs, 1974). Ricklefs in the same paper also stated that house wren nestlings made less effort to maintain their body heat (in terms of number of shivering episodes and percent of times tremors were present) from 32-37.8°C. Average nest temperature for passerine nests recorded by different authors ranged from 34-37°C as reported by Blem (1973). We recorded temperatures within or very slightly greater than the above range in the nest. So it is evident from our daily recordings of ambient temperatures and nest temperatures that throughout our study period the nestlings were in their TNZ.

Gross consumption estimates for the nestling period (13 d for tree swallows, 11 d for house wrens and 15 d for purple martin nestlings) were used to calculate the contaminant accumulation by nestlings as per the equation

$$\Delta BB = (C \cdot BW \cdot [Pc] \cdot ASSIM) / CD_t,$$

the other parameters needed are the contaminant content in the diet of each species, the caloric density of the food items and the absorption efficiency of the nestlings for different contaminants. Daily accumulation could be calculated with appropriate parameters.

Diet contaminant contents are determined by GC/MSD. The caloric density of insect diet for tree swallow and purple martin nestlings was set as 1.24 kcal/g wet wt. (Nichols *et al.*, 1995; 2004), and for house wrens the diet value was 0.85 kcal/g wet wt. (Dauwe *et al.*, 2006). The absorption efficiency was selected as 90% from Drouillard

and Norstrom (2000) who suggested a range of values from 86 to 93% and fat absorption efficiency of 90%.

Growth measurements

To determine growth rates, we measured individual nestlings everyday from several boxes from the day of hatching to almost fledging age. Tree swallows were weighed until 16 days of age, purple martins until their 20th days, and wrens until the 13th day. We used a triple beam balance for measuring the nestlings in the field to the nearest 0.1 g. We weighed the nestlings at approximately the same time every day to maintain consistency in measurements. The nestlings were marked with coloured cable ties to identify them.

Data analysis

Analysis of Variance (ANOVA) was used to determine the significance of differences in concentrations of Σ DDT and PCB in the eggs, diets and tissues of nestlings of each species. Fisher LSD (least square difference) method was used to compare the means to quantify the differences among three species. The significance level was set at $p < 0.05$. All analyses were conducted using Statistica[®] version 6.1 software (Statsoft, Inc., 1997). All data sets were log transformed before statistical analysis as the variances in some groups were not homogeneous. All contaminants were expressed in ng/g wet weight (ww). Σ PCB concentration is the sum of the concentrations of the 38 congeners (IUPAC #: 18, 17, 31/28, 33, 52, 49, 44, 74, 70, 95, 101, 99, 87, 110, 82, 118, 105, 151, 149, 153/132, 138, 158, 128, 156, 187, 183, 177, 171, 180, 191, 170, 201, 195, 194, 205, 208, 206 and 209) analyzed and quantified in each egg, diet and nestling tissue sample. p,p'-DDT, p,p'-DDE and p,p'-DDD are added up to get Σ DDT.

There were differences in the accumulation different PCB and organochlorines based on their retention rate in the tissues. So based on the studies by Drouillard *et al.*, (2001; 2007) and Clark *et al.*, (1987) we grouped PCB congeners and other organochlorines into three groups. This was based on the number of open *meta-para* positions on their biphenyl ring and their half life in different organisms. Open *meta-para*

positions in their biphenyl rings make them more easily attacked by metabolic processes and eliminated from animal tissues.

Rapidly Cleared (half lives of 10-60 d): OCS, dieldrin, PCB 31/28, 52, 49, 44, 70, 95, 101, 87, 110, 1511, 149.

Intermediate (half lives >60-150 d): HCB, PCB 74,

Slowly Cleared (half lives > 150 d): oxychlordane, mirex, DDE, PCB 99, 105.118, 153/132, 138, 158, 128, 156, 171, 177, 178, 180, 170, 183, 187/182, 195, 194, 201, 205, 206, 208, 209.

The bioenergetics based predictions for each congener was done for each species. Then the congeners were separated into readily cleared congener group and slowly cleared group. In this study we grouped the PCB congener with intermediate clearance rate along with the slowly cleared congener group. The predicted and observed values for each species for two different congener groups were then compared using paired Student's t tests. If there were significant differences between predicted and observed values especially in readily cleared congeners would be a measure of their biotransformation and elimination. To evaluate the K_{ow} related accumulation patterns linear regressions were performed with each PCB congener accumulated and predicted (independent variables) against K_{ow} of congeners (dependent variable).

Results

Growth: The three species exhibited considerable interspecific differences in hatching weights, asymptote weight and number of days required to reach asymptote weight (Table 1).

The logistic equation

$$\text{mass } (m) = \text{Asymptote body weight } (A) / (1 + (B) * \exp((-k) * \text{Age}))$$

(where B and k are regression constants) fitted observed growth in the field, well (Fig. 1,2,3 and 4). All three species showed standard sigmoid curves as the weight of the nestlings increased to approximately the adult level during the nestling period (Ricklefs, 1968; 1984). We calculated the hatching weight as a percentage of adult weight and

also determined the time required to grow from 10% to 90% of asymptotic body mass to compare the growth of these species with other species studied in the field (Table 1).

The growth curve of house wren nestlings ($n = 10$ and for 14 days of growth) described by the logistic curve (Figure 1 and 2), gave an asymptotic body mass estimate of 10.305 g, and the constant parameters B and k were 8.452 and 0.555. The time required to grow from 10% to 90% of asymptotic body mass was 8 d. For purple martin nestlings ($n = 18$ and for 21 d of growth, Figure 1 and 3), the asymptotic body mass was 57.04 g and B and k were 12.078 and 0.377, and the time required to grow from 10% to 90% of asymptotic biomass was 13.5 d. For tree swallow nestlings ($n = 10$ and for 15 d of growth, Figure 1 and 4), the asymptotic body mass was 24.92 g, the constant parameters B and k were 12.723 and 0.402, and the time required to grow from 10% to 90% of asymptotic body mass was 11.5 d.

Total contaminant accumulation: The accumulation of Σ DDT was not significantly different among species ($F = 1.07$, $p = 0.3$, HW $n = 5$, PM $n = 4$, TS $n = 4$) (Figure 5)) even though the diet of purple martin nestlings had a significantly lower concentration of Σ DDT than other two ($F = 7.5$, $p = 0.04$, HW $n = 3$, PM $n = 2$ and TS $n = 2$) (Figure 6) and a lower concentration in their eggs ($F = 6.64$, $p = 0.01$, HW $n = 4$, PM $n = 5$, TS $n = 4$) (Figure 7) (Table 2).

The accumulation of Σ PCB among species was significantly different ($F = 42.82$, $p < 0.00001$, HW $n = 5$, PM $n = 4$, TS $n = 4$) with lowest value observed in house wrens and highest in tree swallows (Figure 8). The eggs of purple martins had significantly lower accumulation levels than other two species ($F = 22.86$, $p = 0.0002$, HW $n = 4$, PM $n = 5$, TS $n = 4$) (Figure 9) but the diet of house wrens had a significantly lower Σ PCB than other two species ($F = 9.68$, $p < 0.05$, HW $n = 3$, PM $n = 2$ and TS $n = 2$) (Figure 10) (Table 2).

Bioenergetics model: The diet and egg concentration values (mean) along with the estimated consumption values of each species were used to predict the DDE (DDE was the most abundant chemical found in all nestling tissues and were detected in all other

Table 1. Observed hatching weight and growth parameters estimated by logistic regression of mean daily weight (see Figures 1-4).

Species	Mean±SE Hatching weight (g)	Asymptotic body mass (g)	Hatching weight as % of asymptote	Parameter B	Growth constant k	Days needed to grow 10 to 90% of asymptote weight
House wren	1.43±.09 (n=3)	10.305	13.87	8.452	0.555	8
Purple martin	3.9±.1 (n=3)	57.039	6.83	12.078	0.377	13.5
Tree swallow	1.66±.05 (n=10)	24.923	6.66	12.723	0.402	11.5

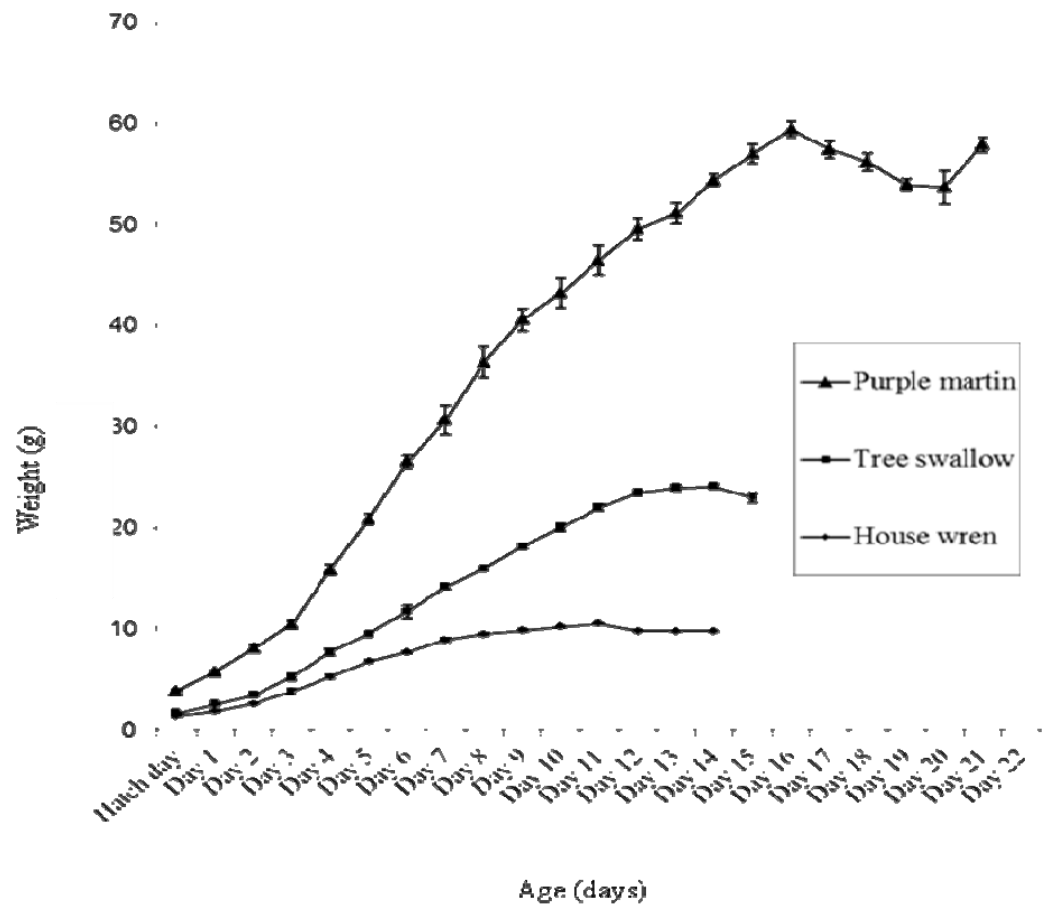


Figure 1. Comparison of observed growth of three passerine nestlings. Lines are fitted through the means \pm SE of each day's measurements

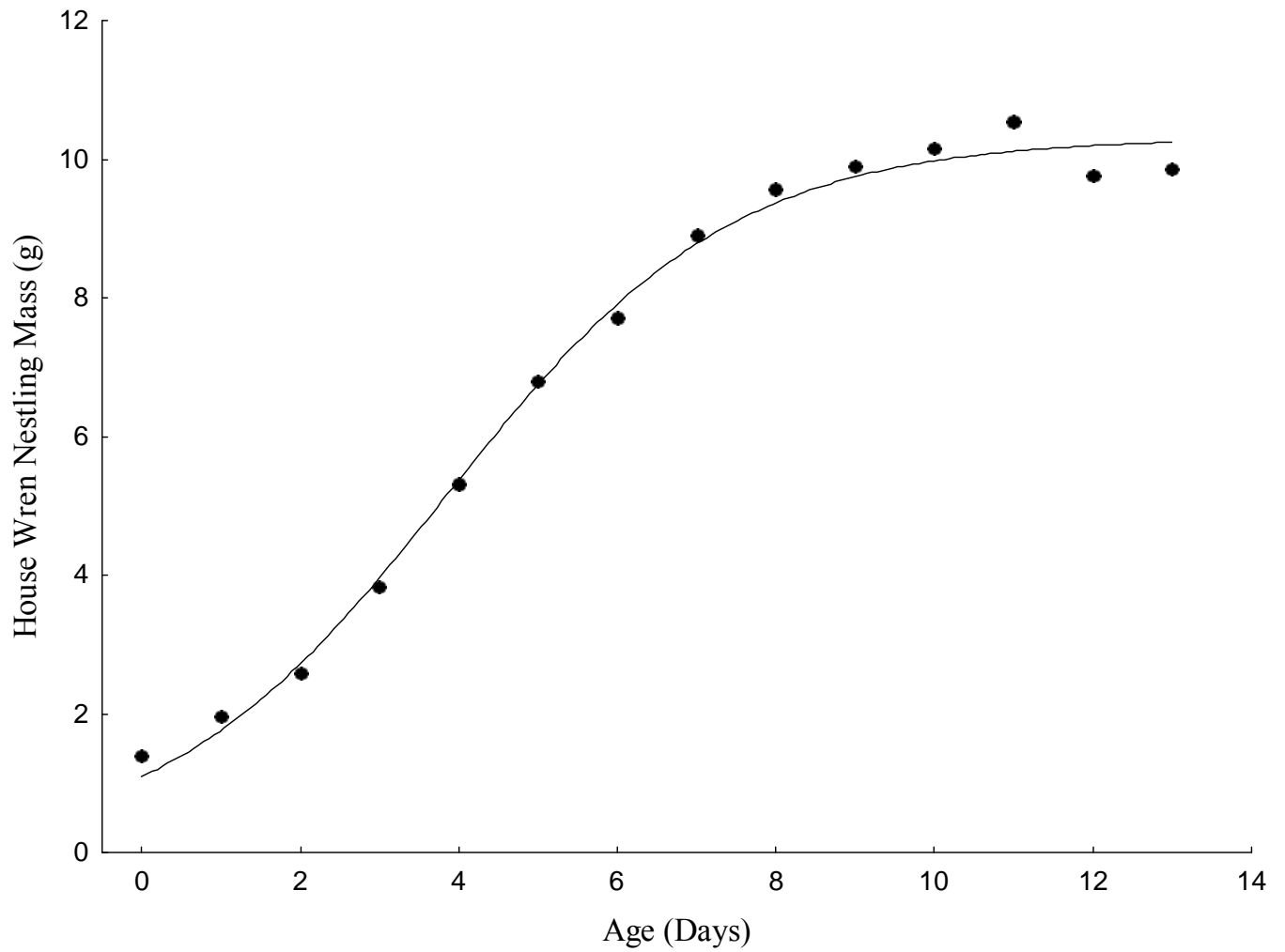


Figure 2. Growth of house wren nestlings showing mean of measured nestlings' mass on each day as individual points and growth curve fitted by nonlinear regression. Regression equation takes the form $\text{Mass} = 10.305 / (1 + (8.452 * e^{-0.555 * \text{age}}))$, $R^2 = 0.88$.

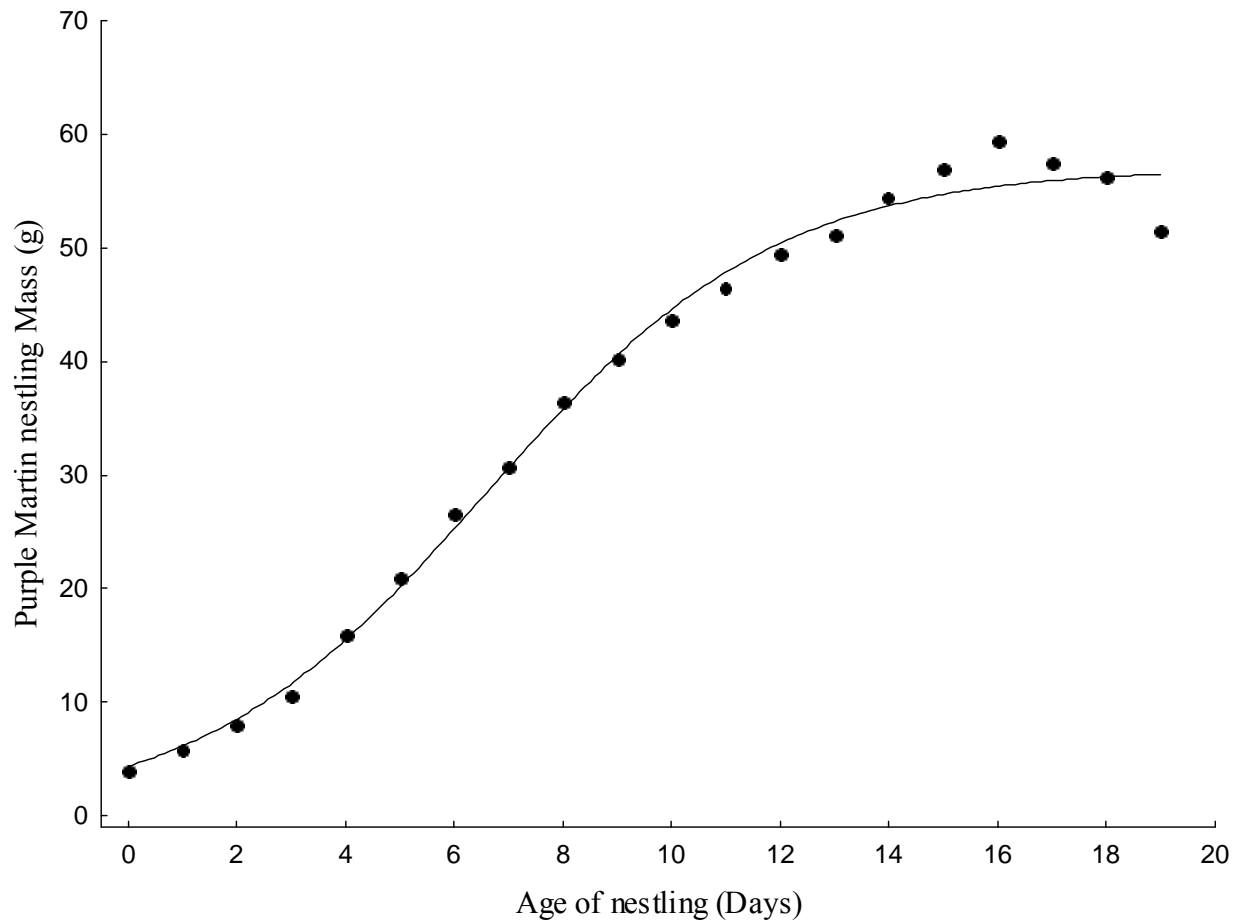


Figure 3. Growth of purple martin nestlings showing mean of measured nestlings' mass on each day as individual points and growth curve fitted by nonlinear regression. Regression equation takes the form $\text{Mass} = 57.039 / (1 + (12.078 * e^{-0.377 * \text{age}}))$, $R^2 = 0.92$.

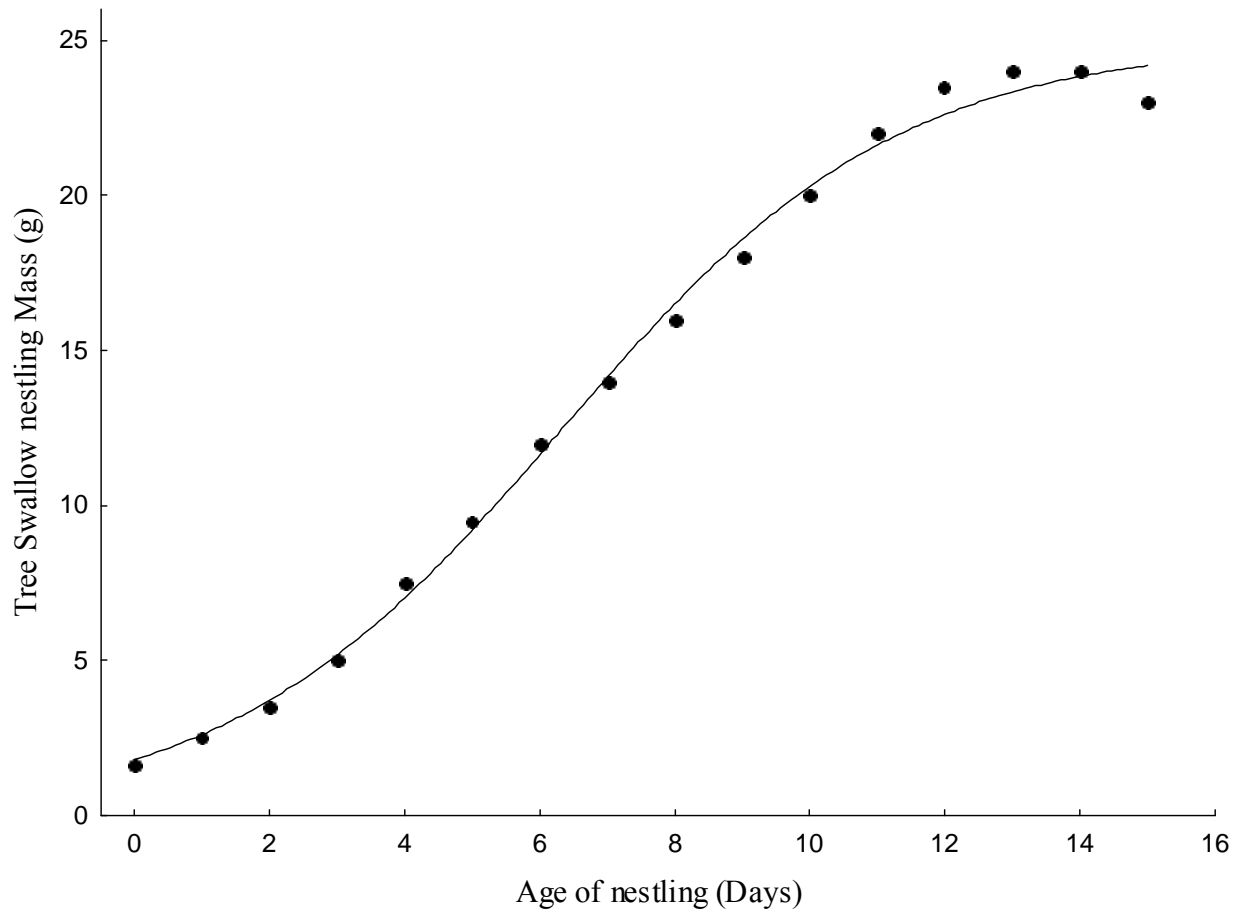


Figure 4. Growth of tree swallow nestlings showing mean of measured nestlings' mass on each day as individual points and growth curve fitted by nonlinear regression. Regression equation takes the form $\text{Mass} = 24.923 / (1 + (12.723 * e^{-0.402 * \text{age}}))$, $R^2 = 0.96$.

Table 2. Mean \pm SE Σ DDT and Σ PCB in eggs and diet of 3 three species.

Species	Mean \pm SE ΣDDT in eggs ng/g	Mean \pm SE ΣDDT in diet ng/g	Mean \pm SE ΣPCB in eggs ng/g	Mean \pm SE ΣPCB in diet ng/g
House wren	334.64 \pm 93.9 (n=4)	30.56 \pm 10.17 (n=3)	2631.52 \pm 561.02 (n=4)	24.45 \pm 9.55 (n=3)
Purple martin	202.63 \pm 140.48 (n=5)	10.04 \pm .9 (n=2)	567.13 \pm 292.63 (n=5)	101.87 \pm 4 (n=2)
Tree swallow	639.74 \pm 116.6 (n=4)	14.76 \pm 3.03 (n=2)	1824.63 \pm 270.6 (n=4)	258.58 \pm 148.75 9n=20

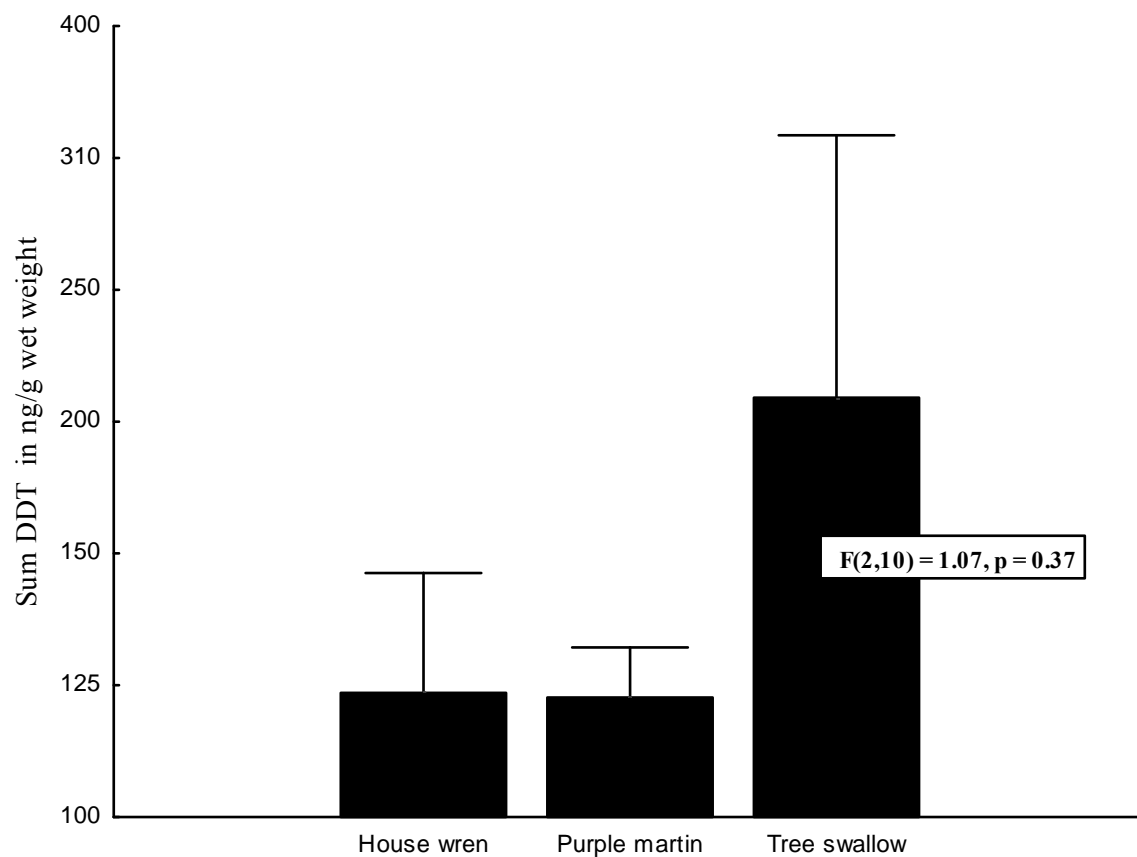


Figure 5. Mean \pm SE Σ DDT accumulation by the nestlings of three species

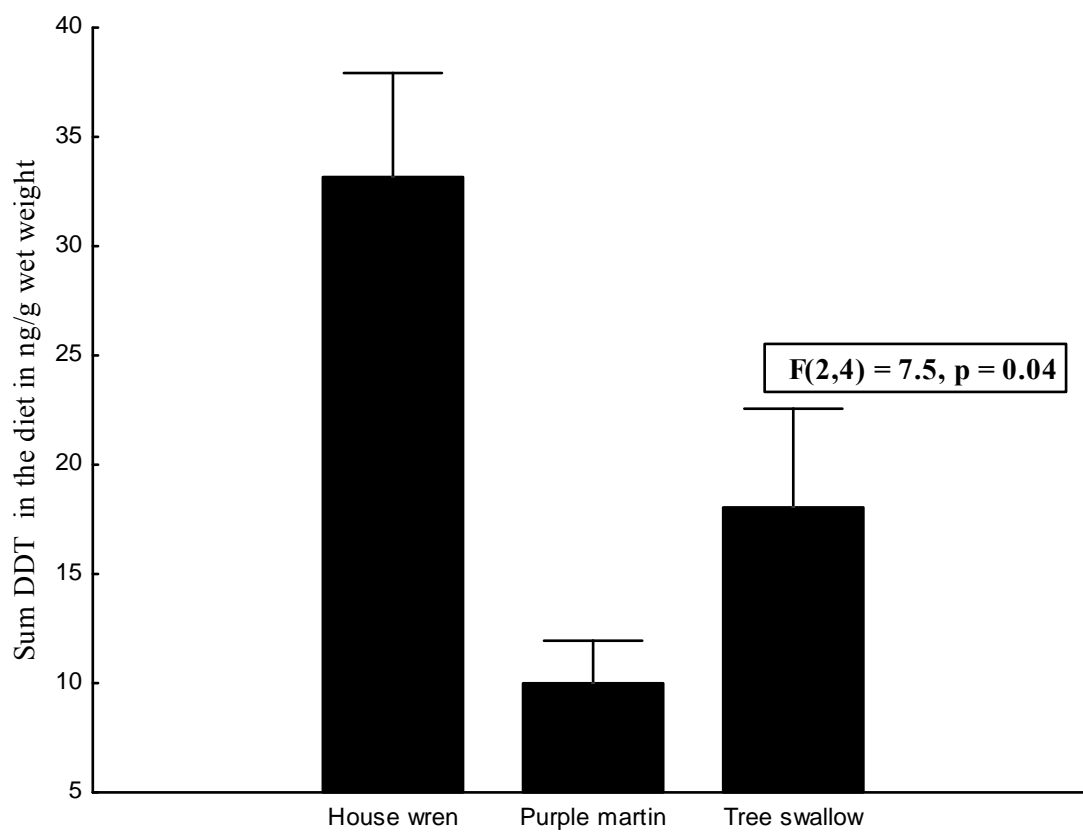


Figure 6. Mean \pm SE Σ DDT accumulation in the diet of the nestlings of three species

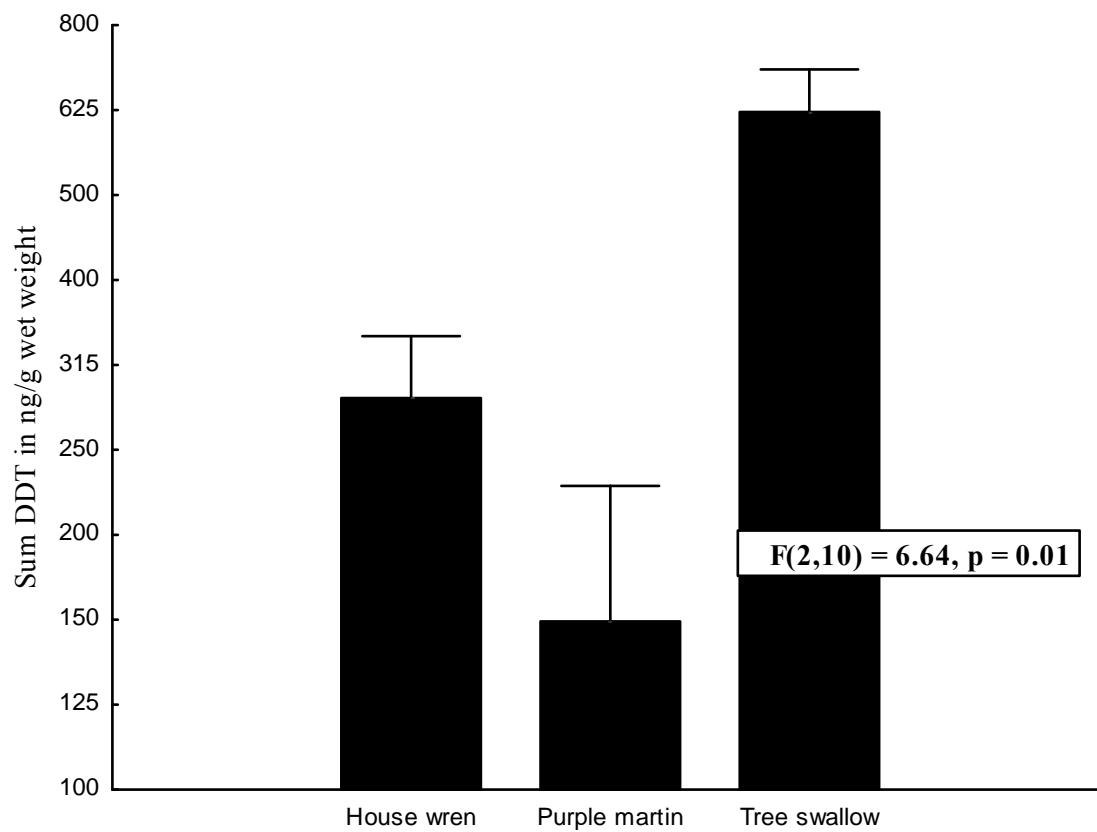


Figure 7. Mean \pm SE \sum DDT accumulation in the eggs of three species

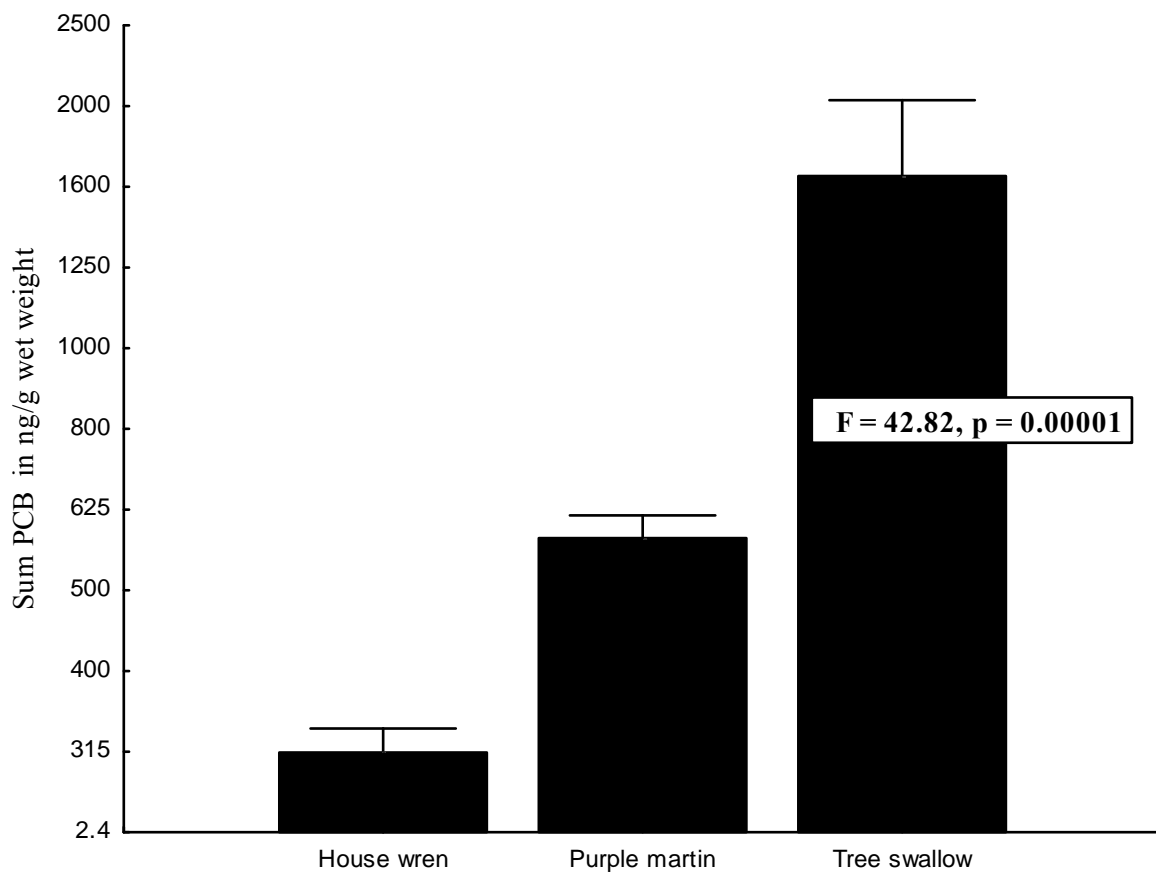


Figure 8. Mean \pm SE Σ PCB accumulation by the nestlings of three species

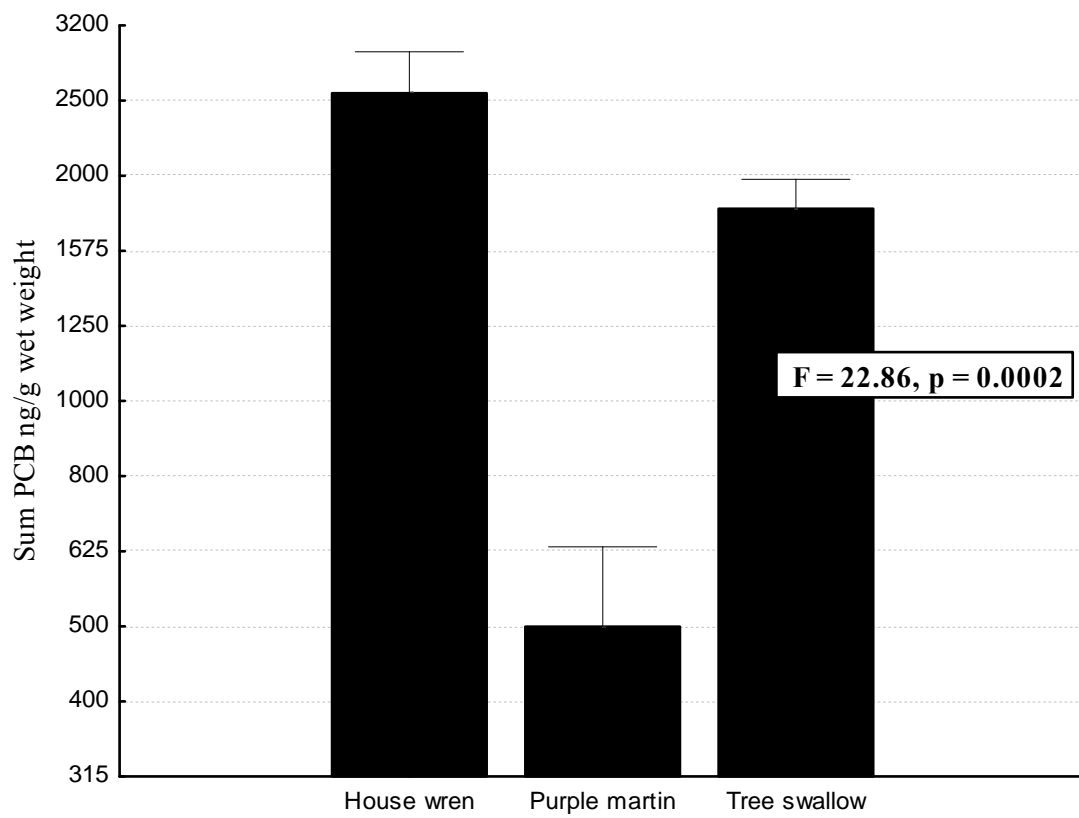


Figure 9. Mean \pm SE Σ PCB accumulation in the eggs of three species

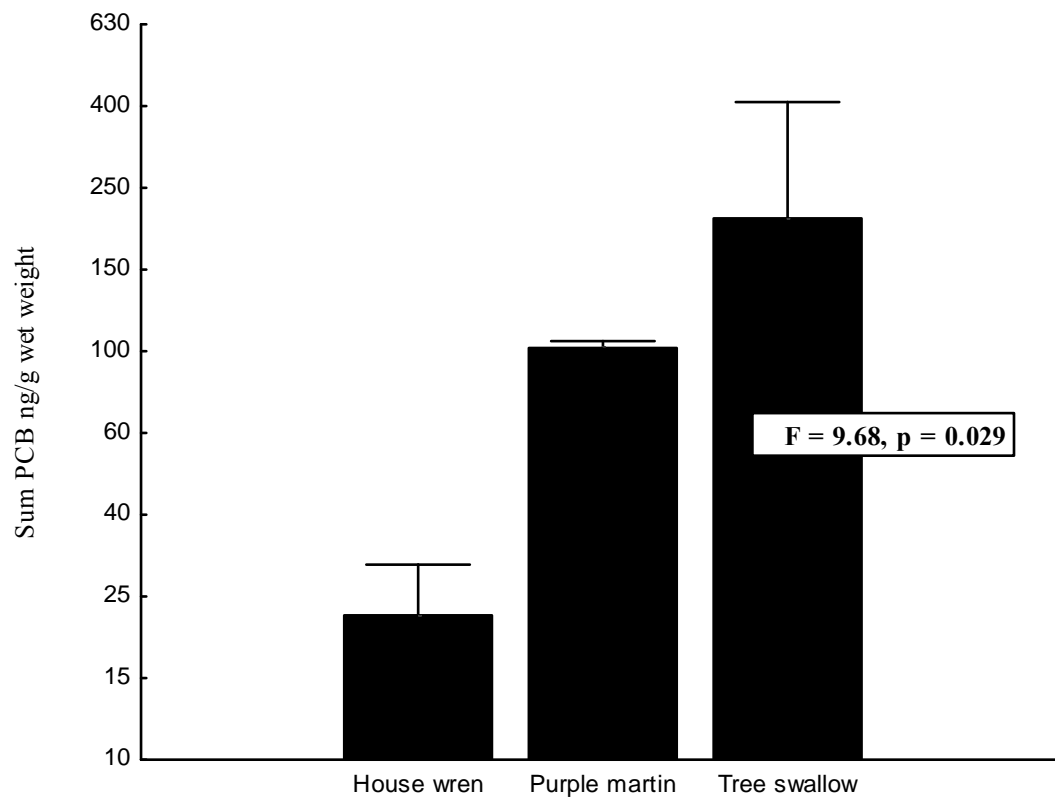


Figure 10. Mean \pm SE Σ PCB accumulation in the diet of the nestlings of three species

samples) and each PCB congener's concentration was used to predict congener specific PCB accumulation in the tissues of nestlings (Tables 3-8). Regression analyses were conducted with observed and predicted values (Figures 11 and 12) for both readily cleared and slowly cleared congeners. PCB congeners that were detected in more than 50% of the samples analysed were used for the predictions. All values are expressed in ng/g wet weight. The regression equations showed that for readily cleared congeners the predicted values were close to ideal situation for house wrens and tree swallows but was overpredicted for purple martins. For slowly cleared congeners tree swallows and purple martins were overpredicted but the house wren PCB concentrations was underpredicted. The predicted value of DDE for house wren nestlings fell well within the range of observed values, whereas for purple martin nestlings, the observed values were above the predicted value. For tree swallow nestlings, two of the observed values were higher than the predicted value and two were very close to the predicted value (Figure 13).

The predicted values for house wren nestlings for readily cleared congeners was significantly lower than the observed values (Student's t test $p < 0.05$) (Figure 14) and for purple martin nestlings the predicted values were significantly higher than observed values (Student's t test $p < 0.0001$) (Figure 15) and there was no significant difference between predicted and observed values in tree swallow nestlings (Student's t test $p > 0.05$) (Figure 16). For slowly cleared congeners both purple martin nestlings (Student's t test $P < 0.01$) (Figure 17) and tree swallow nestlings (Student's t test $p < 0.01$) (Figure 18) were significantly different between predicted and observed values. There was no significant difference between predicted and observed values (Student's t test $p > 0.05$) (Fig. 19) in house wren nestlings.

K_{ow} based accumulation pattern for readily cleared congeners for both predicted and observed values showed significant positive correlation for purple martin nestlings (predicted values against K_{ow} : $r = 0.78$, $p < 0.01$ and observed values: $r = 0.87$, $p < 0.001$) and for tree swallow nestlings (predicted against K_{ow} : $r = 0.63$, $p < 0.05$ and observed against K_{ow} : $r = 0.74$, $p < 0.01$). For house wren the predicted values were

significantly correlated (predicted against K_{ow} : $r = 0.87$, $p < 0.001$) whereas the observed values showed no significant correlation (observed against K_{ow} : $r = 0.37$, $p > 0.05$). When congeners 31/28 and 101 (two outliers) were removed from the analysis the correlation became significant (observed against K_{ow} : $r = 0.72$, $p < 0.05$) (Figure 20).

The relationship between slowly cleared congener group accumulation patterns based on K_{ow} values showed a negative trend in all cases. None of the correlations were significant. In the case of house wren nestling both predicted (predicted against K_{ow} : $r = -0.09$, $p > 0.05$) and observed (observed against K_{ow} : $r = -0.2$, $p > 0.05$) (Fig. 19) were not significantly correlated. In purple martin and tree swallow nestlings also observed the same relationship (purple martin predicted against K_{ow} : $r = -0.23$, $p > 0.05$; observed against K_{ow} : $r = -0.35$, $p > 0.05$ (Fig. 15) and tree swallow predicted against K_{ow} : $r = -0.18$, $p > 0.05$; and observed against K_{ow} : $r = -0.14$, $p > 0.05$).

Discussion

Despite prohibition of the import, manufacture, and sale of PCBs in Canada in 1977 and the ban on their release into the environment in 1985 (Environment Canada, 2006), and the complete ban of DDT from Canada in 1985 (Pesticide News, 1998), the tissues of insects and birds in Point Pelee National Park had detectable amounts of those chemicals in their tissues. Even though they were accumulating persistent organochlorine contaminants in their eggs and the diet, the nestlings' daily absolute growth rates were not impaired.

The growth constant (k) for house wren nestlings with an asymptote mass of 10.305 g was 0.555 which is 11% higher than the value of 0.464 predicted by Ricklefs (1968) for the same species. The asymptotic mass of 10.305 g was 7.5% less than Ricklefs' (1968) value and the number of days required to grow from 10% to 90% was faster than his values, which suggests that the nestlings were growing at a fast rate in the park. Shortening of nestling period can be due to many reasons. This was the second clutch, as egg-laying began after mid July (Johnson, 1998). When there are two or more clutches, nestling period tends to be shortened to exploit the optimal weather conditions available. There was severe predation in the park, which possibly serves as a selection factor for

Table 3. Mean \pm SE concentration of PCB congeners (readily cleared) and DDE ng/g wet weight in eggs (n=4) and diet samples (n=3) and tissues (n=5) and predicted values in house wrens (HW)

Chemicals	HW egg mean\pmSE	HW food mean\pmSE	Predicted	Observed mean \pmSE
PCB 31/28	1.98 \pm 1.12	0.28 \pm 0.07	1.87	11.49 \pm 7.93
PCB 52	0.79 \pm 0.2	0.37 \pm 0.15	2.38	2.99 \pm 2.24
PCB 49	1.88 \pm 1.16	0.28 \pm 0.1	1.90	3.83 \pm 2.64
PCB 44	0.29 \pm 0.03	0.21	1.31	1.27 \pm 1.38
PCB 70	1.36 \pm 0.37	0.27 \pm 0.06	1.78	5.73 \pm 5.19
PCB 95	1.17 \pm 0.32	0.41 \pm 0.23	2.62	3.38 \pm 2.77
PCB 101	31.16 \pm 19.27	0.66 \pm 0.26	6.04	10.02 \pm 4.91
PCB 87	13.14 \pm 4.45	0.32 \pm 0.12	2.81	5.37 \pm 3.05
PCB 110	23.6 \pm 15.6	0.66 \pm 0.27	5.58	10.03 \pm 5.07
PCB 151	1.82 \pm 1.3	0.32 \pm 0.11	2.13	2.17 \pm 0.96
PCB 149	56.32 \pm 21.96	0.93 \pm 0.4	9.24	10.14 \pm 3.22
DDE	310.09 \pm 91.29	20.73 \pm 12.7	148.72	111.71 \pm 36.02

Table 4. Mean±SE concentrations of PCB congeners (slowly cleared) ng/g wet weight in eggs (n=4) and diet samples (n=3) and tissues (n=5) and predicted values in house wrens (HW)

PCB congeners	Egg mean±SE	Food mean±SE	PCB predicted	PCB observed mean ±SE
PCB 74	14.44±4.39	0.18±0.03	1.98	5.43±3.37
PCB 99	44.71±10.03	0.78±0.35	7.60	6.5±2.52
PCB 118	125.9±15.85	0.93±0.31	13.49	17.55±7.74
PCB 105	46.95±6.22	0.36±0.04	5.09	16.12±9.49
PCB 3/132	498.17±102.62	4.58±1.66	58.87	35.45±20.77
PCB 138	356.05±79.75	2.54±0.87	37.48	33.43±12.37
PCB 158	23.9±3.22	0.18±0.08	2.55	2.81±0.73
PCB 128	46.7±9.08	0.41±0.15	5.37	4.8±1.76
PCB 156	34.78±5.37	0.32±0	4.11	3.47±1.16
PCB 187	153.38±28.48	1.52±0.6	18.81	12.6±7.45
PCB 183	77.62±12.99	0.45±0.25	7.52	5.42±2.93
PCB 177	44.04±5.89	0.47±0.11	5.63	4.04±1.7
PCB 171	53.03±32.3	ND	0.00	1.26±1.25
PCB 180	475.98±86.89	3.98±1.45	53.78	33.33±18.89
PCB 170	194.37±32.57	1.38±0.52	20.44	13.24±6.66
PCB 201	85.73±15.12	0.84±0.43	10.45	6.83±3.67
PCB 195	25.26±3.14	0.12±0	2.30	1.55±0.81
PCB 194	149.12±35.9	1.19±0.39	16.51	10.11±5.52
PCB 205	2.33±1.66	ND	0.00	0.85±0.48
PCB 208	7.83±2.13	0.23±0	1.93	1.08±0.86
PCB 206	38.64±11.4	0.33±0.07	4.44	2.54±1.43

Table 5. Mean \pm SE concentrations of PCB congeners (readily cleared) and DDE ng/g wet weight in eggs (n=5) and diet samples (n=2) and tissues (n=4) and predicted values in purple martins

Chemicals	Egg mean\pmSE	Food mean\pmSE	Predicted	Observed mean \pmSE
PCB 31/28	2.01 \pm 1.95	1.05 \pm 0.02	7.05	1.92 \pm 0.36
PCB 52	1.76 \pm 0.89	2.61 \pm 0.05	17.45	6.50 \pm 0.92
PCB 49	1.73 \pm 1.32	1.54 \pm 0.08	10.33	3.62 \pm 0.85
PCB 44	0.020 \pm 0.03	1.28 \pm 0.19	8.53	1.42 \pm 1.04
PCB 70	2.41 \pm 0.82	1.53 \pm 0.01	10.27	2.27 \pm 0.26
PCB 95	2.77 \pm 1.33	3.64 \pm 0.01	24.34	4.18 \pm 2.31
PCB 101	14.42 \pm 7.92	5.83 \pm 0.12	39.43	24.51 \pm 3.69
PCB 87	3.16 \pm 1.14	2.49 \pm 0.48	16.73	6.17 \pm 1.68
PCB 110	11.38 \pm 5.30	5.92 \pm 0.08	39.92	13.01 \pm 5.38
PCB 151	4.85 \pm 2.48	1.91 \pm 0.08	12.91	3.05 \pm 1.07
PCB 149	23.17 \pm 11.93	5.73 \pm 0.02	39.16	23.78 \pm 12.54
DDE	182.31 \pm 127.25	8.43 \pm 0.26	64.09	115.31 \pm 133.09

Table 6. Mean±SE concentrations of PCB congeners (slowly cleared) ng/g wet weight in eggs (n=5) and diet samples (n=2) and tissues (n=4) and predicted values in purple martins

PCB congeners	Egg mean±SE	Food mean±SE	Predicted	Observed mean ±SE
PCB 74	2.75±0.85	1.13±0.24	7.62	3.10±1.04
PCB 99	10.45±7.29	3.23±0.25	21.96	11.50±4.78
PCB 118	20.0±7.41	3.88±0.32	26.70	42.21±28.72
PCB 105	6.90±2.22	3.09±2.13	20.84	34.46±46.40
PCB 153/132	95.44±41.66	16.17±0.24	111.80	98.34±21.41
PCB 138	80.21±36.52	11.82±0.30	82.19	80.75±9.20
PCB 158	8.95±8.93	0.60±0.23	4.42	3.99±2.15
PCB 128	10.07±4.54	1.91±0.02	13.14	12.93±1.22
PCB 156	5.03±1.25	0.82±0.12	5.69	5.78±0.65
PCB 187	34.50±16.17	3.04±0.33	21.77	26.41±4.80
PCB 183	21.74±15.37	1.71±0.07	12.31	15.34±7.05
PCB 177	14.21±9.31	1.50±0.36	10.61	14.61±7.33
PCB 171	5.55±2.02	0.70±0.06	4.91	10.24±6.06
PCB 180	87.83±43.71	9.01±0.19	63.82	72.21±16.90
PCB 170	37.66±15.81	4.50±0.19	31.60	28.99±9.33
PCB 201	22.52±12.60	1.03±0.19	7.85	10.79±0.92
PCB 195	7.66±4.10	0.46±0.09	3.37	5.21±1.03
PCB 194	23.36±5.59	2.30±0.01	16.32	21.97±3.59
PCB 208	2.57±1.53	0.18	1.28	0.69±0.22
PCB 206	2.38±3.69	0.45	3.07	3.85±0.93

Table 7. Mean \pm SE concentrations of PCB congeners (readily cleared) and DDE ng/g wet weight in eggs (n=4) and diet samples (n=2) and tissues (n=4) and predicted values in tree swallows

Chemicals	Egg mean\pmSE	Food mean\pmSE	Predicted	Observed tissue mean \pmSE
PCB 31/28	4.59 \pm 0.50	7.90 \pm 3.43	31.09	13.23 \pm 7.00
PCB 52	9.51 \pm 1.13	7.85 \pm 2.78	31.16	40.76 \pm 26.09
PCB 49	3.21 \pm 0.52	3.75 \pm 1.04	14.82	20.99 \pm 13.50
PCB 44	0.33 \pm 0.24	4.27 \pm 1.33	16.67	11.66 \pm 7.76
PCB 70	6.28 \pm 0.93	4.71 \pm 1.69	18.74	23.93 \pm 8.18
PCB 95	9.17 \pm 1.03	11.10 \pm 3.43	43.83	50.35 \pm 17.80
PCB 101	29.06 \pm 3.20	13.89 \pm 4.22	55.81	58.48 \pm 57.18
PCB 87	8.63 \pm 1.01	6.28 \pm 1.88	24.99	24.17 \pm 15.90
PCB 110	26.71 \pm 3.37	14.52 \pm 4.48	58.12	52.47 \pm 50.67
PCB 151	10.81 \pm 1.26	4.49 \pm 1.11	18.12	20.67 \pm 15.58
PCB 149	58.20 \pm 6.58	14.79 \pm 4.08	60.87	68.06 \pm 61.16
DDE	615.73 \pm 113.55	11.77 \pm 2.22	84.37	217.82 \pm 133.09

Table 8. Mean \pm SE concentrations of PCB congeners (slowly cleared) ng/g wet weight in eggs (n=4) and diet samples (n=2) and tissues (n=4) and predicted values in tree swallows

PCB congeners	Egg mean\pmSE	Food mean\pmSE	Predicted	Observed tissue mean \pmSE
PCB 74	7.48 \pm 0.86	2.72 \pm 0.88	11.02	15.04 \pm 8.07
PCB 99	17.08 \pm 2.05	6.35 \pm 1.53	25.71	28.54 \pm 23.15
PCB 118	58.89 \pm 6.53	10.19 \pm 3.36	42.98	195.23 \pm 133.84
PCB 105	25.79 \pm 3.00	3.56 \pm 1.28	15.29	40.03 \pm 8.39
PCB 153/132	276.37 \pm 31.95	35.39 \pm 9.41	153.12	309.26 \pm 88.32
PCB 138	244.17 \pm 33.14	27.72 \pm 7.27	121.45	254.44 \pm 74.35
PCB 158	16.36 \pm 2.56	2.22 \pm 0.64	9.55	12.78 \pm 7.29
PCB 128	29.24 \pm 4.60	4.23 \pm 0.91	18.10	33.85 \pm 6.05
PCB 156	18.84 \pm 2.61	2.06 \pm 0.63	9.06	13.98 \pm 8.21
PCB 187	126.33 \pm 19.66	7.29 \pm 1.92	35.29	69.33 \pm 32.34
PCB 183	57.92 \pm 7.48	4.84 \pm 1.22	22.03	31.84 \pm 25.84
PCB 177	37.09 \pm 8.73	3.65 \pm 0.98	16.26	31.51 \pm 8.05
PCB 171	18.32 \pm 5.19	1.68 \pm 0.52	7.55	23.65 \pm 12.55
PCB 180	341.36 \pm 51.39	20.84 \pm 4.79	99.83	147.26 \pm 147.52
PCB 170	140.93 \pm 24.35	10.13 \pm 2.62	47.17	124.43 \pm 31.18
PCB 201	72.39 \pm 15.06	3.37 \pm 0.88	17.08	55.94 \pm 29.98
PCB 195	22.75 \pm 2.98	1.64 \pm 0.42	7.65	16.84 \pm 3.98
PCB 194	110.06 \pm 35.93	6.31 \pm 1.66	30.57	76.80 \pm 13.27
PCB 208	6.23 \pm 0.80	0.30	1.52	1.85 \pm 1.17
PCB 206	27.55 \pm 6.00	1.70 \pm 0.33	8.12	10.47 \pm 6.01

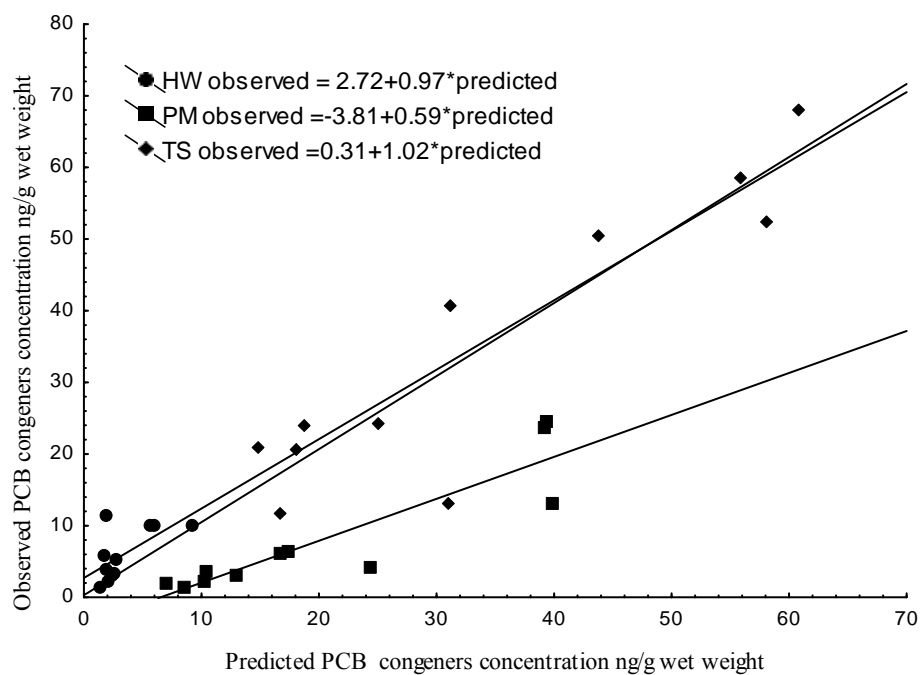


Figure 11. Predicted and observed concentrations of readily cleared PCB congeners for three species

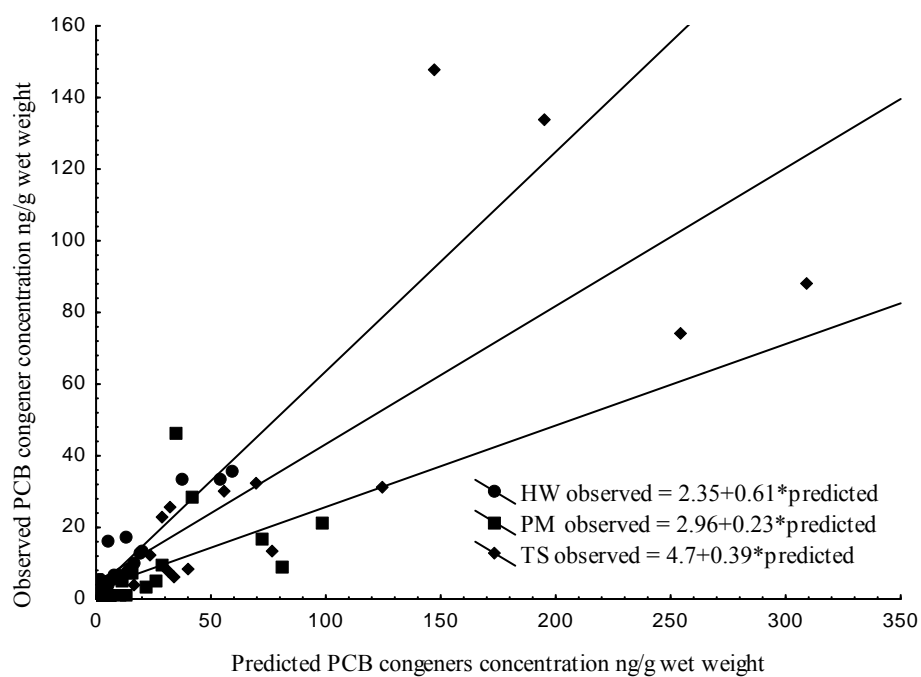


Figure 12. Predicted and observed concentrations of slowly cleared PCB congeners for three species

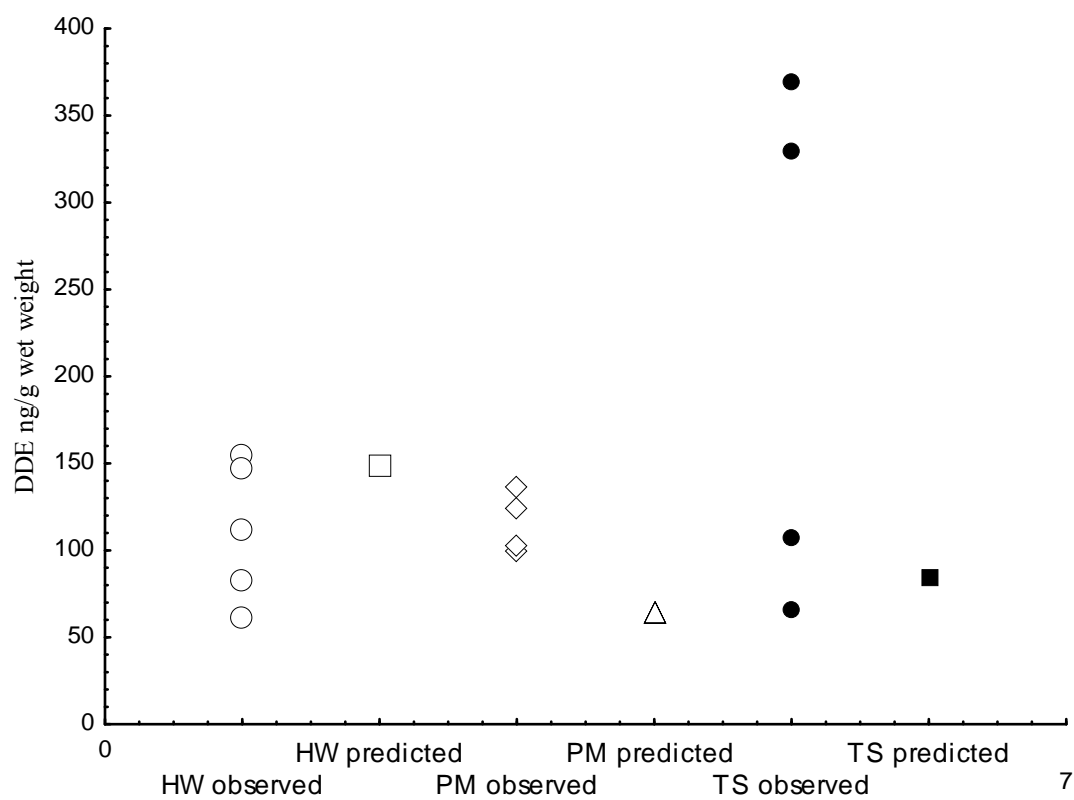


Figure 13. Predicted and observed values of DDE in ng/g wet wt. in the nestling tissues of three species

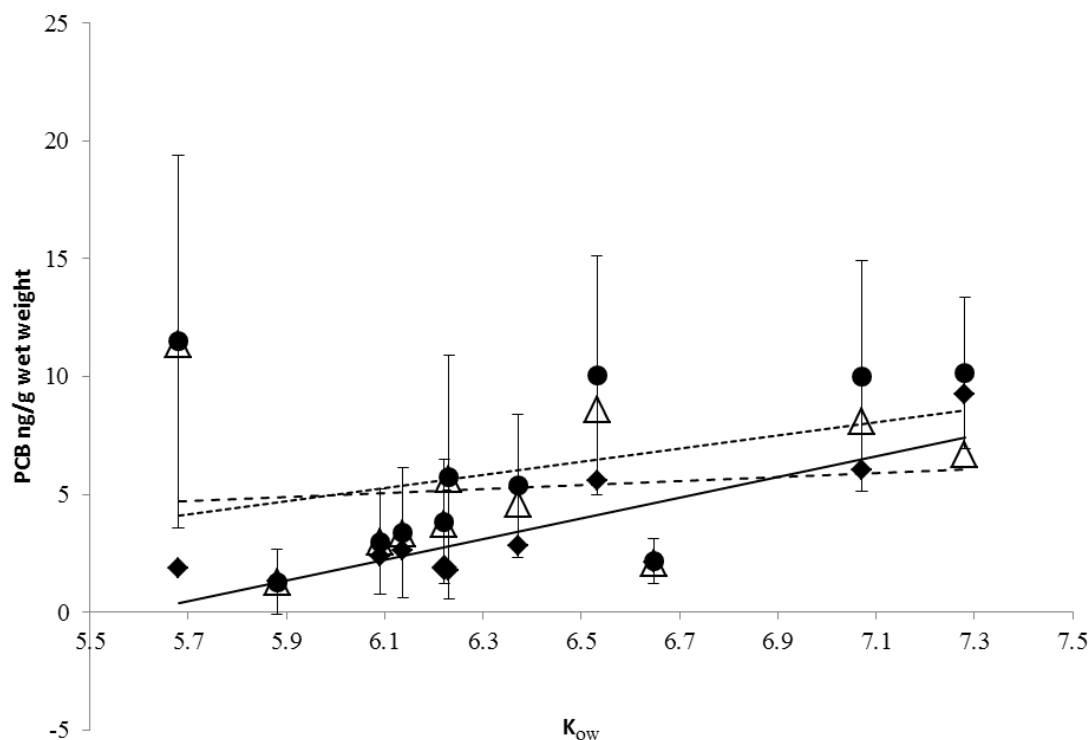


Figure 14. Predicted (♦) and observed (•) mean \pm SE and contribution from the breeding site (Δ) values of readily cleared congeners and linear regression lines PCB predicted (—), PCB observed (.....) and PCB from the breeding site (-----) in house wren nestlings for 10 days of age (observed vs. predicted $p < 0.05$) (predicted against K_{ow} : $r = 0.87$, $p < 0.001$; observed against K_{ow} : $r = 0.37$, $p > 0.05$)

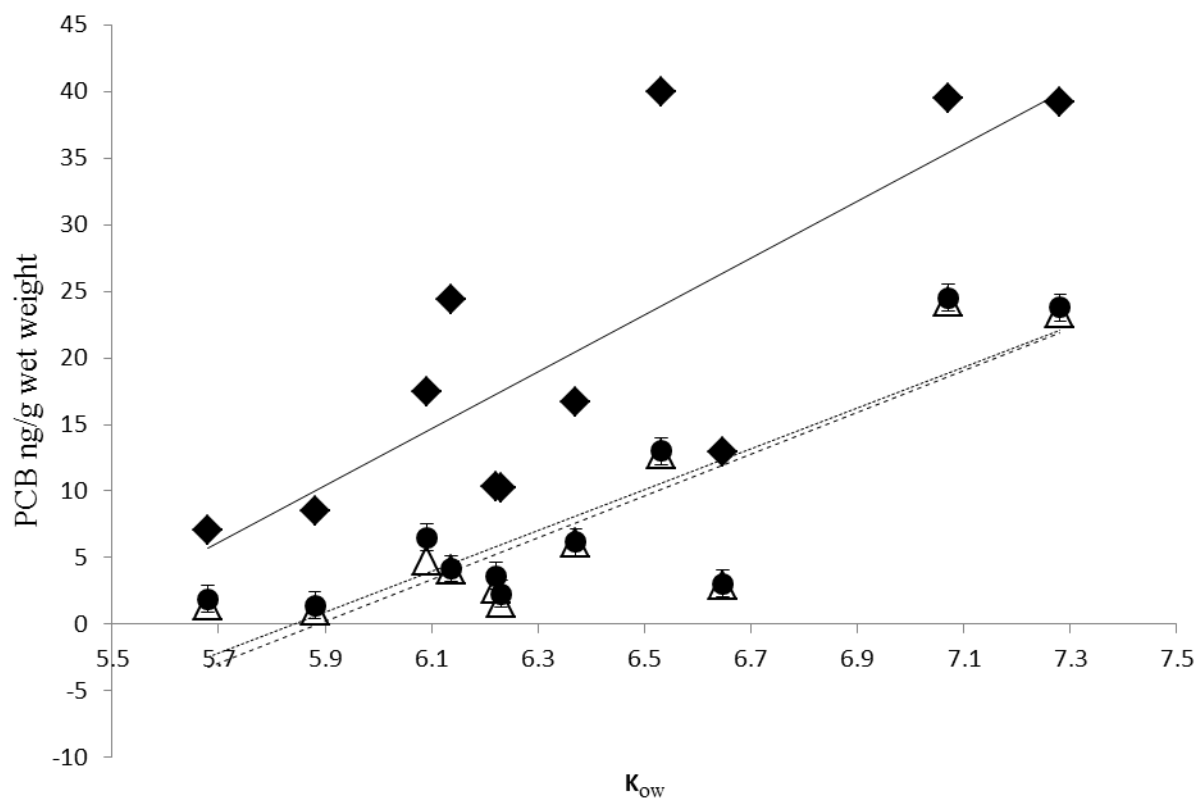


Figure 15. Predicted (♦) and observed (•) mean \pm SE and contribution from the breeding site (Δ) values of readily cleared congeners and linear regression lines PCB predicted (—), PCB observed (.....) and PCB from the breeding site (-----) in purple martin nestlings for 15 days of age (observed vs. predicted $p < 0.0001$) (predicted values against K_{ow} : $r = 0.78$, $p < 0.01$; observed values: $r = 0.087$, $p < 0.001$)

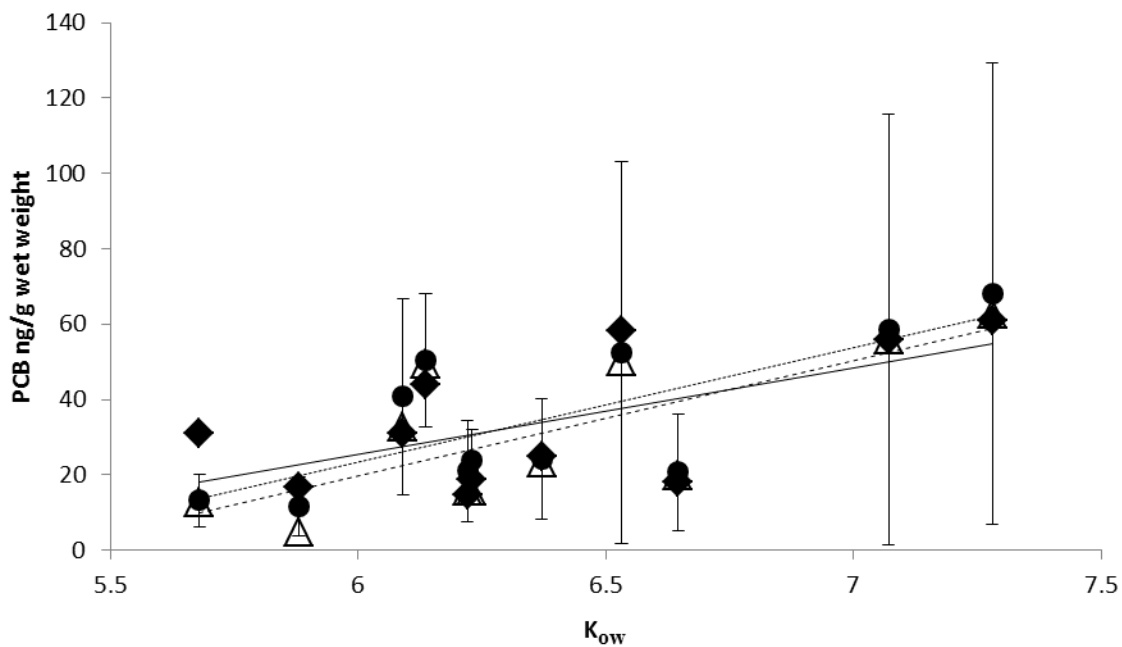


Figure 16. Predicted (♦) and observed (•) mean \pm SE and contribution from the breeding site (Δ) values of readily cleared congeners and linear regression lines PCB predicted (—), PCB observed (.....) and PCB from the breeding site (-----) in tree swallow nestlings for 13 days of age (observed vs. predicted $p > 0.05$) (predicted against K_{ow} : $r = 0.63$, $p < 0.05$ and observed against K_{ow} : $r = 0.74$, $p < 0.01$)

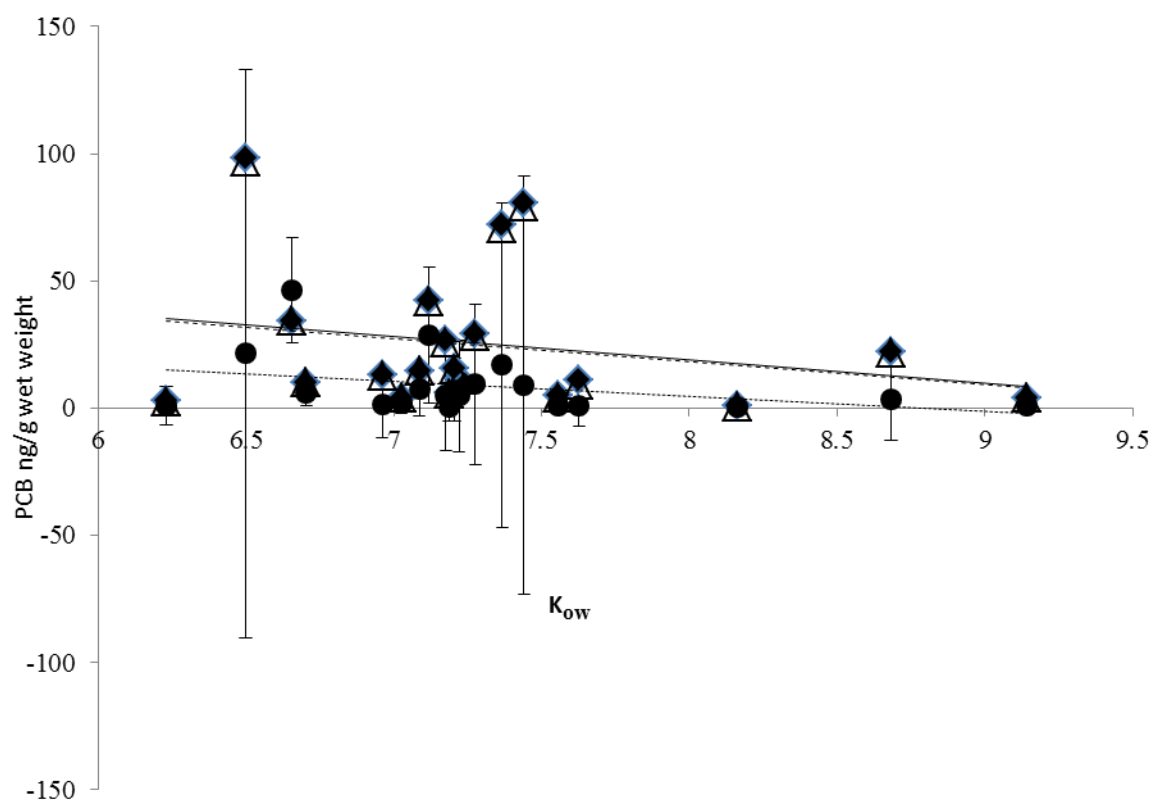


Figure 17. Predicted (\blacklozenge) and observed (\bullet) mean \pm SE and contribution from the breeding site (Δ) values of slowly cleared congeners and linear regression lines PCB predicted (—), PCB observed (.....) and PCB from the breeding site (-----) in purple martin nestlings for 15 days of age (observed vs. predicted $p < 0.01$) (predicted against K_{ow} : $r = -0.23$, $p < 0.05$; observed against K_{ow} : $r = -0.35$, $p > 0.05$)

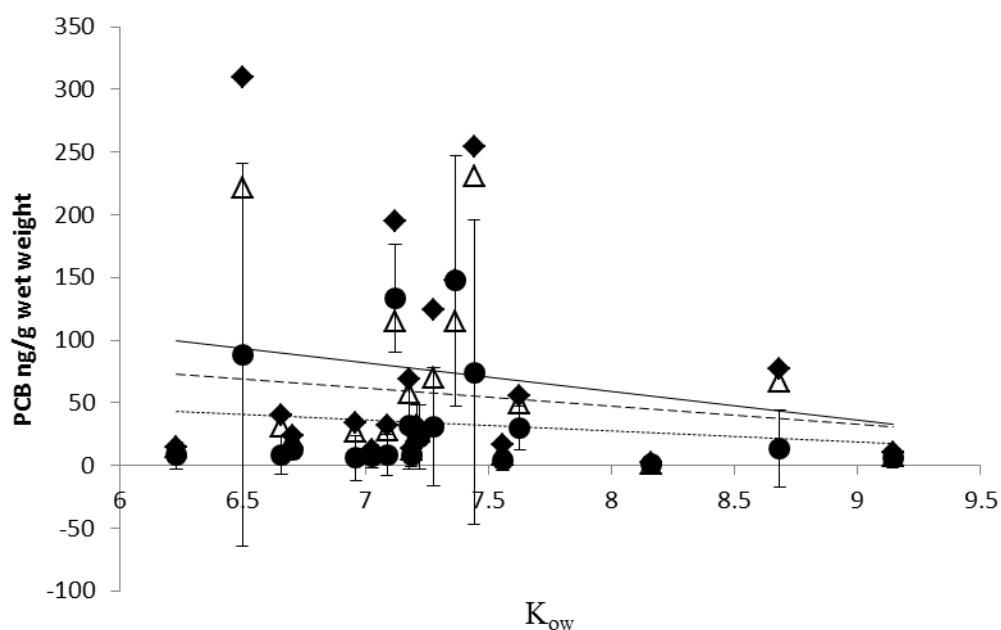


Figure 18. Predicted (♦) and observed (•) mean \pm SE and contribution from the breeding site (Δ) values of slowly cleared congeners and linear regression lines PCB predicted (—), PCB observed (.....) and PCB from the breeding site (-----) in tree swallow nestlings for 13 days of age (observed vs. predicted $p > 0.01$) (predicted against K_{ow} : $r = 0.63$, $p < 0.05$; observed against K_{ow} : $r = 0.74$, $p < 0.01$)

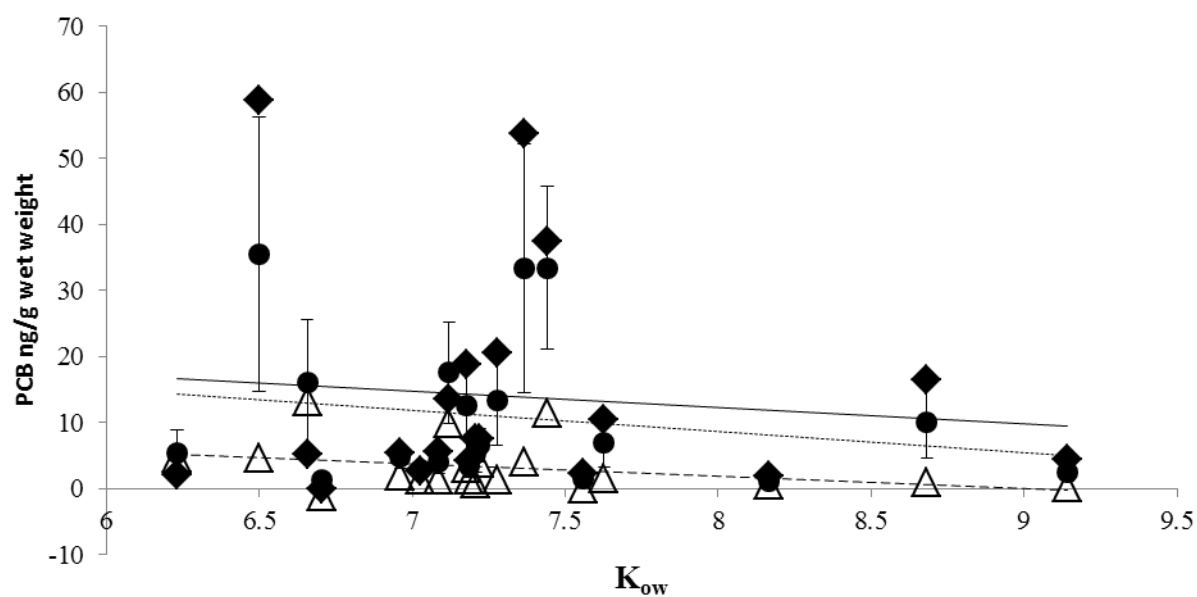


Figure 19. Predicted (♦) and observed (•) mean \pm SE and contribution from the breeding site (Δ) values of slowly cleared congeners and linear regression lines PCB predicted (—), PCB observed (.....) and PCB from the breeding site (-----) in house wren nestlings (observed vs. predicted $p > 0.05$) (predicted against K_{ow} : $r = -0.09$, $p > 0.05$; observed against K_{ow} : $r = -0.2$, $p > 0.05$)

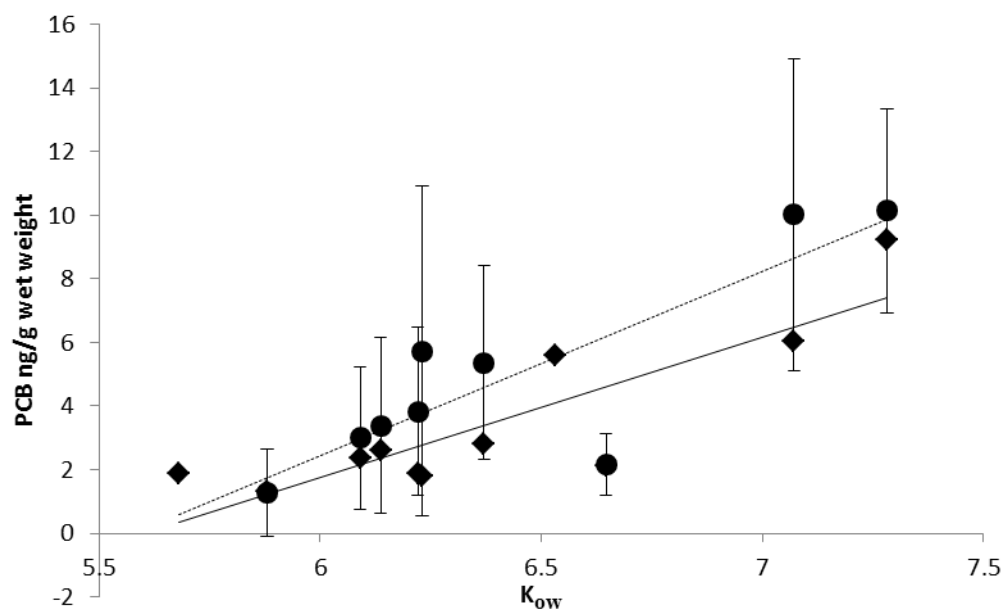


Figure 20. Predicted (♦) and observed (•) mean \pm SE (with PCB congener 31/28 and 110 removed) values of readily cleared congeners and linear regression lines PCB predicted (—), and PCB observed (.....) in house wren nestlings for 10 days of age (observed vs. K_{ow} $r=0.72$, $p<0.05$ and predicted vs K_{ow} $r=0.87$, $p<0.001$)

Table 9. Contribution of different PCB congeners from egg (percent) to the tissue concentration in three species

Readily cleared congeners	House wren	Purple martin	Tree swallow	Slowly cleared congeners	House wren	Purple martin	Tree swallow
PCB 31/28	1.01	5.61	2.06	PCB 74	15.51	4.75	2.96
PCB 52	1.54	1.45	1.68	PCB 99	40.12	3.64	3.56
PCB 49	2.86	2.54	1.19	PCB 118	41.85	1.90	2.91
PCB 44	1.32	0.08	0.41	PCB 105	16.99	0.80	4.58
PCB 70	1.39	5.66	2.27	PCB 153/132	81.99	3.89	6.64
PCB 95	2.02	2.66	1.08	PCB 138	62.14	3.98	5.71
PCB 101	18.14	2.36	2.96	PCB 158	49.60	8.98	7.62
PCB 87	14.28	2.05	2.12	PCB 128	56.75	3.12	5.94
PCB 110	13.72	3.50	3.03	PCB 156	58.45	3.48	8.02
PCB 151	4.89	6.38	3.11	PCB 187	71.03	5.24	10.84
PCB 149	32.40	3.91	5.09	PCB 183	83.47	5.68	10.82
				PCB 177	63.62	3.90	7.01
				PCB 171	147.03	2.17	4.61
				PCB 180	83.31	4.87	13.79
				PCB 170	85.63	5.21	10.11
				PCB 201	73.18	9.39	7.70
				PCB 195	95.15	7.82	12.66
				PCB 194	86.05	4.26	8.53
				PCB 205	19.91	0.00	12.15
				PCB 208	42.26	20.04	19.98
				PCB 206	88.70	2.48	15.66

fast growth. Rapid development to fledging minimizes predation risk, permitting individuals to move to other new areas with lesser risk (Anava *et al.*, 2001). This higher growth rate in the park suggests that these birds maximize the use of resources available in the habitat by an “optimal time budgeting” (Ricklefs, 1969).

For purple martin nestlings the asymptote body mass of 57.039 g was about 5% less than the predicted value for the same species by Ricklefs (1968), and the k value of 0.377 was about 2% less than he predicted. These differences in the case of purple martin nestlings were negligible, and are well within the normal range of development across the geographic range of the species (Ricklefs, 1968).

The tree swallows’ asymptote mass of 24.923 g and k of 0.402 and 11.5 d to grow from 10 to 90% of asymptote weight were in agreement with other Hirudinidae predicted by Ricklefs (1968).

The growth rate of an organism will be affected by many factors, including food resources, clutch size, food available to the clutch, food available to each individual in the clutch, total metabolizable energy and energy available for growth (Ricklefs, 1969). The energy required for growth is determined by the physiology and biochemical state of the developing organism and also the parental care offered to the individuals especially in altricial birds. The values obtained for growth of the 3 study species reveal that the growth of these passerine nestlings was not limited by physiological or ecological constraints that are often encountered in natural habitats. The growth parameters k and asymptote weight, along with the time required to grow from 10 to 90% of asymptote weight are very useful in intraspecific and interspecific comparisons. We followed the nestlings until fledging and found that unless preyed upon (there was severe raccoon predation in the park), they developed normally after completing their standard prefledging period. We did not notice any pollution-related reproductive impairment in any of the three species studied.

The model gave reasonable predictions for the accumulation of DDE and PCB congeners even though in certain cases the predicted values exceeded the observed levels or some cases fell below the observed levels. When it is below predicted level it could be attributed to biotransformation/elimination processes. When it is above the observed levels it could be due to many reasons such as differences in feeding rates, chemical and

lipid absorption efficiencies, intestine and food partition coefficient, fugacity capacities of different compartments etc. (Kelly *et al.*, 2004). The model predictions could be improved by increasing the sample size to permit the egg and the diet values to have been estimated with greater precision. These were the least well-estimated elements of our model. We avoided trying to change the parameters derived from literature to improve the models' fit with the observed data. Instead, the goal of this study was to see the extent to which the nestlings' bioenergetics could be used to investigate the contaminant accumulation pattern.

Some of the assumptions we made (e.g., assimilation efficiency of contaminants at 90%) can also vary, depending on the growth phase of the nestling, weather conditions and nature of the diet items, especially the lipid content and the individual's physiological condition. Drouillard and Norstrom (2003) found that PCB uptake by birds fed on a low lipid diet was significantly higher than in birds fed on high lipid diet even though their assimilation efficiencies were the same. Nichols *et al.*, (2004) used a value of 0.70 to predict accumulation for one of their study sites and 0.72 for another site to get a better fit for their model.

When we assessed the onsite accumulation based on the contaminant content of the food eaten and the load acquired from the egg, each species showed different levels of onsite accumulation and biotransformation capabilities. The contribution from diets (egg contribution subtracted from the observed contaminant content in the tissues) for each species for each congener group were included in figures 14-19 for comparison with observed values to visually summarize the magnitude of onsite accumulation in each species.

House wren nestlings showed very little onsite accumulation, especially in the case of slowly cleared congeners. Most of these nestlings' contaminants were transferred from the eggs (Table 9). House wrens' diet consisted of terrestrial insects (Skutch 1953; Neigh *et al.*, 2006; Chapter 3). The study by Smits *et al.* (2005) and analyses described in Chapter 3 found that terrestrial insects in Point Pelee National Park were depleted of PCBs compared to aquatic insects. So site specific accumulation was minimised in this species. As they retained most of the egg contaminants it could be assumed that they showed very limited biotransformation capacity. The odd behavior of having a higher

observed PCB concentration especially in the case of readily cleared congeners in house wren nestlings pointed to the fact that the contaminants were likely underestimated either in their eggs or in their diet.

Purple martin nestlings showed onsite accumulation of different PCB congeners. Nestlings exhibited biotransformation capacity similar to adults. This was evident from the significantly lower observed values in this species in the readily cleared congener accumulation. Food contributed a comparatively large proportion of PCBs in this species (Table 9). The egg PCB load was significantly lower in purple martins than in house wrens, so most of the accumulation for this species was from food acquired at the breeding ground.

Tree swallows showed an intermediate pattern compared to other two species. They accumulated most of their contaminants from food collected by their parents on the breeding ground. But they exhibited very low or no biotransformation capacity. The observed and predicted values of the readily cleared congeners showed no significant difference. Studies by Nichols *et al.*, (1995; 2004) and Papp *et al.*, (2007) suggested that tree swallow nestlings possessed very little biotransformation ability. This study also pointed out the same trend. It would be valuable to determine at what age this species develops detoxifying capabilities.

The observed and predicted values of the readily cleared congeners were significantly correlated with their K_{ow} values except for house wrens. In house wrens, congeners 31/28 and 110 were found in higher proportions than other readily cleared congeners. These congeners were also represented in higher amounts in their diet insect samples. The anomalously high concentrations of these two congeners need further investigation to account for their occurrence at higher than expected levels compared to others in the same category. Elimination of readily cleared congeners depends on hydrophobicity and chlorine substitution patterns (Drouillard *et al.*, 2007). Drouillard *et al.*, (2007) assessed the bioaccumulation and biotransformation of 61 PCB congeners in juvenile American kestrels and found that congeners with vicinal hydrogen substitution at *meta-para* carbons on at least one phenyl ring were eliminated relatively quickly, whereas the ones with chlorine substitution at 4,4'-, 3'4,5-, 3,4',5-, or 3,3',5,5-positions showed higher retention in the tissues (ranged 13.2 to 81.5%). The group that was

eliminated at a faster rate and the ones that were retained showed a significant correlation with the K_{ow} of the congener. But in this study the K_{ow} relationship was observed only in the readily cleared congeners. The group of slowly-cleared congeners showed a negative correlation with the K_{ow} values. This negative trend might be due to absorption/assimilation differences exhibited by the congeners depending on their K_{ow} . Dietary assimilation efficiencies were found to decrease from 0.93 to 0.85 with K_{ow} increasing from 5 to 7.5 (Norstrom, 2002). There are not many studies on the biotransformation abilities of juvenile birds other than Nichols *et al.* (1995; 2004), and Drouillard *et al.*, (2007) mentioned above. Drouillard *et al.* (2007) showed that juvenile American kestrels had the same metabolic capability as the adults. But Nichols *et al.*, (1995; 2004) demonstrated with their bioenergetics based model that nestling swallows did not metabolize or eliminate PCB congeners during their prefledging stage of development. Studies on adults of Adelle penguins showed that the half-life for DDT, especially *p,p'*-DDE, for this species was 580 d and PCBs 270 d (Subramanian *et al.*, 1987). Our study species all have very short nestling periods. But American kestrels, which have a juvenile period of only around 28-30 days, showed interestingly complete elimination of most of the readily cleared congeners.

Nestlings with a shorter nestling period also should have the capacity to eliminate pollutants as it is important to deal with pollutants to which they were exposed to at their early life stages. As the nestling period for all three study species are very short, compared to the half lives of the respective pollutants in the above study (Subramanian *et al.*, 1987), elimination through biotransformation and fecal elimination might be following a different pathway. The biotransformation/detoxifying mechanism in fast growing passerine nestlings with very short nestling period should be studied in detail to know the toxicokinetics in their prefledging stage, which are not available in literature. Of the three species, purple martins with the longest prefledging period showed some biotransformation capacity compared to other two study species. It would be interesting to know at which time point in life history they acquire the metabolizing capacity that almost all avian species are assumed to by adulthood (Drouillard *et al.*, 2001).

The model's output also will mainly depend on the accuracy in determining the consumption rate, digestive capability of the organism and determination of the

efficiency in allocation of energy. These values vary in different species and that in turn is the main reason for the interspecific differences in contaminant bioaccumulation (deBruyn and Gobas, 2006).

All three species studied are insectivorous but eat different proportions of aquatic vs. terrestrial insects. They also catch their insects from different spatial niches within the terrestrial habitat. Furthermore, nestlings' species-specific growth rate, their asymptote body mass and (because of it their total consumption) varies, which in turn influences the final total accumulation of the persistent, bioaccumulative chemicals in their tissues.

The Σ PCB accumulation patterns were as expected, with tree swallow nestlings exhibiting the highest values followed by purple martin nestlings, and the lowest in house wren nestlings on a wet weight basis. This expectation was based on the proportion of aquatic insects in the diet of the 3 species. The insects emerging from Lake Erie sediments can have PCBs in their tissues as lake Erie has been contaminated with various amounts of PCBs and other persistent pollutants at different locations from multiple sources (Gewurtz and Diamond, 2003; Marvin *et al.*, 2004; Heidtke *et al.*, 2006). PCB loads in mayflies vary, depending on their emergence location in the lake (Corkum *et al.* 1997).

The accumulation of Σ DDT showed an unexpected trend, with highest accumulation in tree swallow nestlings followed by house wren and purple martin nestlings. This difference in accumulation may be due to the difference in the collection locations of terrestrial insects by swallow parents in the park. There are areas within the park like the DeLaurier site and Camp Henry area where the DDT levels in the soil exceed government guidelines (Smits *et al.*, 2005). The ants collected from DeLaurier site had significantly higher levels of Σ DDT in their tissues than the ants collected from other locations of the park that are designated as uncontaminated areas (Smits *et al.*, 2005). These areas are former apple orchards within the park where DDT was heavily used to control pests and biting insects as mentioned earlier. In the case of PCBs, there were no differences in concentration in insects collected from any location in the park (Smits *et al.*, 2005).

The contaminant loads in the eggs were another determining factor. Tree swallow eggs had a higher load than other two species. The PCB burdens in the tissues of all

nestlings were contributed mainly by the eggs that in turn were a reflection of the maternal burden. The average PCB burden in the egg of house wrens was 2632 ± 561 ng/g ($n=4$) with egg weight 0.63 g (egg weight –shell) (Chapter 3), and the nestlings tissue average was 318 ± 44 ng/g ($n=5$) with nestling weight 10.5 g. So most of the contribution of PCBs to the nestling tissue was from the eggs in this species. We noticed (as have other studies) a growth dilution in nestlings. This was happening because of the noticeably lower contaminant burden in the diet samples. The PCB concentration in the egg could be a reflection of maternal bioaccumulation from either the breeding location in the park or from the overwintering locations (Jayaraman *et al.*, 2009). If the mothers were still retaining contaminants from their prebreeding grounds it casts doubt about the utility of nestlings as indicators for local contamination. This was indicated by Dauve *et al.* (2006) in their study on eggs and nestlings of great tits (*Parus major*). The great tits, in their study, accumulated 67% of PCB congener 153 in 15 day old nestlings' tissues from the eggs.

Another constraint to the model was consumption estimations. The literature provides no clear cut method by which to estimate consumption (Nichols *et al.*, 1995). We used the few estimation methods available that are applicable to field-studies of consumption in passerines of almost similar size and nestling pre fledging duration and selected the most suitable one applicable for all three species.

The bioenergetics-based model can be used as a tool to investigate the accumulation pattern of persistent chemicals and to assess the likelihood that they will build to toxic levels during the pre fledging period. The model also allows investigation of the energetic requirements of the organisms studied. The model predicts contaminant bioaccumulation based on an organism's consumption. Because an individual's consumption during a particular stage of development will be constant, the fluctuating entity in the model prediction is the contaminant content of the diet insects. The insects' contaminant load is determined by the environmental contaminant situation, i.e., the sediment and soil concentrations of the particular contaminants of interest. The insects' bioaccumulation of contaminant depends on biota sediment accumulation factors (BSAF). If the sediment contaminant concentration and BASF factor are known, an accurate bioenergetics based model can

predict the possible bioaccumulation in an organism, and vice versa. The model can then be used to predict the environmental contaminant concentration. The model can also be used to validate many growth and bioenergetics parameters of studied organisms and also to estimate biomagnification and biotransformation of chemicals of concern in study and related organisms. However, the breadth of application of the proposed model is limited by the lack of precision of many of the proposed parameters.

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Chapter 5: Feeding experiments in Point Pelee National Park with passerine nestlings to determine bioaccumulation and to measure morphological and physiological biomarkers

Introduction

Polychlorinated biphenyls (PCBs) and other persistent organochlorine contaminants, even in moderate doses, can modulate the endocrine system and influence vertebrates' normal reproduction and development (Colborn and Smolen, 1996; Dawson, 2000). At high concentrations, these compounds are toxic to many systems including the nervous system and can induce behavioural changes (Walker, 2003), physiological dysfunction (Tanabe, 2002), and modulate immunity (Barron *et al.*, 1995; Fournier, 2000), especially of T cells in wildlife and humans (Grasman and Fox, 2001). These chemicals are capable of mimicking vertebrate steroids, and can interact with steroid receptors (Ottinger *et al.*, 2001). They can be especially harmful during the embryonic, foetal and postnatal periods of development (Fossi, *et al.*, 1999). Because chemical contaminants can affect both morphological and behavioural traits of an organism (McCarthy and Secord, 2000; Bortolotti *et al.*, 2003), these attributes can be used as non-destructive means of assessing the contaminants' presence and effects. Many studies have reported the reproductive and developmental consequences of PCB bioaccumulation and toxicity in birds. One of the most important effects is on overall growth, especially during the nestling period, at which time maximal growth takes place. These can be assessed by daily measurements of mass and by calculating the daily growth of the individual or individual organs. An animal's wellbeing can be evaluated by examining external features such as (in the case of birds) pigmentation of feathers, beak and feet, their general appearance, and their behaviour. External parasite load on individuals or in their nests is another important aspect of health.

The health, physiological status and immunity of animals can also be assessed by measuring hematological and biochemical parameters (Grasman *et al.*, 2000; Sargent *et al.*, 2004). Blood samples can be collected with minimum distress to the animal. Hematological variables that can be used as indicators of immune function and the health status of birds include total red blood cell count, total leucocytes (WBC), % heterophils,

% lymphocytes, % monocytes, % eosinophils and their ratio to one another (Handy and Depledge, 1999; Grasman *et al.*, 2000; 2002).

There are many studies dealing with the developmental effects of endocrine disrupting chemicals such as DDTs and PCBs on wildlife; especially in birds, and many studies in this field focus on bird reproduction and development (Colborn *et al.*, 1993; Barron *et al.*, 1995; Fry, 1995; Hoffman *et al.*, 1986; 1996; Smits and Bortolotti, 2001; Smits *et al.*, 2002; Bustnes *et al.*, 2003; Hario *et al.*, 2004). Most of these studies either measured the effects of naturally accumulating pollutants from the background environment, or were controlled investigations done in the lab using domesticated populations of birds in artificial conditions with spiked food that may or may not have represented natural diet. Some of those previous studies have shown that most birds are resilient to the effects of organochlorine contaminants in small doses. To mitigate these factors, a feeding study in Point Pelee National Park with house wren nestlings was done to introduce different contaminant levels of PCBs and to assess different biomarkers of exposure and effects. We conducted a manipulative study supplementing the diet of wild nestlings with a staple, natural food type (*Hexagenia* mayflies). The concentrations of contaminants in the insects were determined by their bioaccumulation while developing.

In previous years' studies in the park, we found that the soil of areas containing abandoned apple orchards had concentrations of DDT and its metabolites that exceeded guidelines imposed by the government (Smits *et al.* 2005). This soil-associated DDT and its metabolites were bioaccumulated by tree swallow nestlings in locations where the parents fed them locally-collected terrestrial insects. In contrast, tree swallow nestlings developing in park locations where parents fed them aquatic insects had noticeably higher amounts of PCB in their tissues. The Σ PCB in the nestlings' tissues was correlated with the amount of aquatic insects in the diet, especially mayflies (Ephemeroptera mostly *Hexagenia*). The importance of mayflies as a diet item of tree swallow nestlings was confirmed in subsequent studies (Smits *et al.*, 2005 and chapter 2). Consequently, we chose to supplement the normal diet with *Hexagenia* mayflies collected from regions that varied greatly in background levels of PCBs. Our goal was to determine whether differences in PCB concentrations of naturally occurring foods (PCB-contaminated

emergent mayflies) were sufficient to influence nestling development and fledging success in Point Pelee bird populations.

Wrens are swallow-like insectivorous passerines that feed on insects. Wrens build their nests in the same areas as tree swallows. In fact, they are interference competitors because they exclude swallows from boxes by destroying swallow nests (Finch, 1990). Unlike tree swallows, which forage almost entirely by capturing insects in flight, wrens collect insects from the leaves (gleaning). In doing so, they capture and eat many mayflies that rest on to leaves during their landward migration and during transformation from the subimago to the imago stage. Judd (1900) as reported by Gross (1948) identified insects in wren nestlings' diet and found that out of 111 insects and spiders there was: 1 white grub, 1 soldier bug, 3 millers (Noctuidae), 9 spiders, 9 grasshoppers, 15 mayflies and 20 caterpillars. This suggests that wrens in Point Pelee also face the same risk of exposure to PCBs through their diet as tree swallows. House wrens are one of the most common species in Point Pelee National Park

Our objective in this study was to determine bioaccumulation and to assess biomarkers (anatomical, morphological and physiological) for nestling birds exposed to diets containing PCBs and other organochlorines. The bioaccumulation estimates and their potential for causing developmental impairment will be applicable to other insectivorous opportunistic bird species that frequent the park. Gulls in particular feed extensively on spent mayflies following mass swarms. We hypothesized that if a defined proportion of the nestling diet is supplemented with mayflies of known contaminant concentration, then we expect a bioaccumulation depending on bioenergetics principles of the nestlings. We also assumed that there is minimal biotransformation of PCBs in the nestlings (Nichols *et al.*, 1995; 2004).

Materials and methods

Experimental design

Under field conditions, we fed wren nestlings with mayflies containing different concentrations of PCB contamination to induce different levels of bioaccumulation. The planned daily ration was determined using bioenergetics knowledge of nestlings (but for several reasons the amount prescribed to feed couldn't be achieved). The study was

conducted in Point Pelee National Park (see Chapters 2, 3 and 4 for a full description of the study site).

Mayfly collection

Mayflies belonging to the genus *Hexagenia* (mainly *Hexagenia limbata* (Serville) (Ephemeroptera: Ephemeridae)) were collected from 3 locations that varied greatly in background concentrations of PCBs. The sediment-dwelling larvae of mayflies bioaccumulate persistent organic compounds and trace metals during their 1-2 years of development. Mayflies are peculiar in having a two winged adult stages- an imago and a subimago. The adult stage lasts from one to two hours to a few days, during which they do not feed. They survive on the reserves accumulated during their nymphal stage. The nymphal stage varies from few weeks to about 2 - 2 ½ years depending on water temperature and other water conditions. Mayflies spend most of their life in the aquatic habitat feeding and burrowing in the sediments as nymphs (Brittain 1982; Cibrowski and Corkum, 1988). The emergent adults are attracted to lights at night and in swarms they settle on the ground and on vegetation during their peak emergence period. Huge numbers can be collected by hand at this resting time.

We collected mayflies in the summer months of 2004 by light trapping and hand picking. We collected several hundred g of mayflies from each of three locations 1. Gimli Harbour (50.630486° N, 96.984960° W) of Lake Winnipeg, Manitoba (presumed to be relatively uncontaminated by PCBs), 2. Leamington Marina (42.041046° N, 82.606490° W) on Lake Erie east of Point Pelee, Ontario (moderately contaminated with PCBs) and 3. Sterling State Park (41.907935° N, 83.335778° W) on Lake Erie at Monroe, Michigan (highly contaminated with PCBs). Adults were collected beneath street lights or from a white sheet placed beneath a light trap. The mayflies were placed in a container with dry ice at the time of collection. They were stored frozen at -20°C until they were used for feeding experiments.

Mayflies were collected from these sites with the assumption that the body burdens of the adult flies will be considerably different at different sites. Rawn *et al.* (2000) reported that yearly PCB flux into Lake Winnipeg sediments were from 5 to 11 µg/m², with burdens of 388 and 337 µg/m² in the north and south basins, respectively.

But for Lake Erie there were higher sediment loads from different sources. Noticeable differences in PCB concentration in mayflies collected from different locations of Lake Erie and associated rivers were recorded in studies by Ciborowski and Corkum (1988) and by Corkum *et al.* (1997). Corkum *et al.* (1997) estimated the concentrations of 42 PCB congeners and found higher concentrations of those congeners in the Monroe region of Lake Erie compared to many other locations. Their measurements of concentrations of PCB 101, 138, 153 and 180 in mayflies from Monroe were 3-10fold greater than concentrations in mayflies collected north of Point Pelee. The concentration of PCB 101 for Monroe 786 µg/ kg lipid, and for north of Point Pelee it was 275 µg /kg lipid. For PCB 138 it was 1304 for Monroe and 428 for north of Point Pelee location, for PCB 153 it was 1078 and 426 and for PCB 180 the values were 1024 and 35 for respective sites.

Mayfly preparation

We fed the nestlings with one of four food supplement treatments. Three treatments consisted of the mayflies collected from the three different locations. The fourth treatment consisted of Lake Winnipeg mayflies (the least contaminated sample) spiked with a mixture of PCBs. To a 400-g aliquot of mayflies, we added 1 mL of safflower oil spiked to 8 mg/mL Aroclor 1248: 1254: 1260 mixture. This was done by dissolving the Aroclor mixture in 200 mL of dichloromethane (DCM). The mayflies were placed in a porcelain tray and then soaked with the PCB-spiked DCM solution and allowed to air dry under a fume hood. The other mayfly treatments were soaked in an equal quantity of unspiked DCM to ensure that all of the diet samples were of consistent texture and quality. The prepared mayflies were weighed into 1.5-g fractions (daily rations), which were stored individually in small glass bottles until needed.

Establishment of wren colony

We erected 21 nest boxes in the marsh boardwalk area of the park (Appendix Fig. 5). We monitored all the nest boxes daily (a sample sheet is provided, Appendix Table 1) and randomly selected five nest boxes in which 6 or more eggs were laid as replicates for the study (box # 1, 4, 11, 15 and 21). One egg was collected from each experimental nest box after the egg laying period had ended. We used a randomized block design to allocate

nestlings to food supplement treatments. Nest boxes were treated as blocks, and each one of five nestlings in a box was assigned to a different treatment. Four treatments consisted of a nestling being fed with one of four mayfly supplements. The remaining treatment was an unmanipulated control. The one nestling fed with Winnipeg mayflies (minimum PCB) was considered as a manipulated control. Five individuals were selected from each nest box and were randomly tagged with cable ties of different colours. The diet samples were kept in containers with the same colour as the colour of the nestling to which it was fed. The nestlings that were fed with spiked sample were **reds**; those fed mayflies collected from Monroe, Michigan were **yellows**; those fed mayflies from the Point Pelee area were **greens**; the ones that were fed mayflies collected from Lake Winnipeg (manipulated controls) were **blues** and the unmanipulated controls were **whites**.

Data for this study were collected throughout one breeding season (May to August 2005). Nestling mass over 12 d post hatching (measured to the nearest 0.1 g) was used as an index of growth (McCarthy & Secord, 1999). Measurements were taken every day. We measured tarsal length, ninth primary feather length, quill length of primary feather, bill length (all with calipers), wing chord and tail length (with a ruler) to estimate the growth of nestlings (Harris and Elliott, 2000; Smits *et al.*, 2000; Kuzyk, *et al.*, 2003) (Table 1).

Each bird and nest site was examined daily for the presence of ectoparasites. In particular, the wing pits of nestlings were examined before each feeding. We sampled the nest cavities by inserting our hands to the nest cup wearing white gloves. At the end of the experimental period, we collected nest materials after the nestlings had been removed from the boxes. These were stored frozen in plastic bags. Subsequently, the materials were placed on sieves of different mesh sizes and shaken. Fine materials passing through the sieve were carefully examined for associated ectoparasites.

On the day of necropsy, we qualitatively recorded the amount of subcutaneous fat (migratory fat) on each nestling, as another indication of their condition (Proctor and Lynch, 1993). Fat deposits could easily be seen by blowing back the feathers of the breast and looking at the notch between clavicle bones. This was classified on a scale of 0 to 3

Table 1. Data sheet (of box# 21) showing the measurements of nestlings during feeding experiment from July 18th to 25th 2004.

Date		Spiked	Monroe	Point Pelee	Lake Winnipeg	Control
Jul-18	Weight (g)	9.1	8.6	10	10.8	8.4
Jul-20		8	8.4	9	9.3	7.2
Jul-22		8.7	7.8	9.2	10	9
Jul-25		8.2	Dead	9.2	10.5	9.07
Jul-18	Wing chord (mm)	12	18	21	24	12
Jul-20		22	22	26		19
Jul-22		26	25	31	33	28
Jul-25		31		34	40	34
Jul-18	Pri. Feather (mm)	8	8	10	10	5
Jul-20		10	9	10		8
Jul-22		9	11	12	11	12
Jul-25		12		17	19	16
Jul-18	P.F- Quill (mm)	0	0	1	0	0
Jul-20		1	1	3		0
Jul-22		3	4	5	8	3
Jul-25		7		12	13	7
Jul-18	Tail (mm)	4	3	5	7	3
Jul-20		6	6	11		4
Jul-22		9	6	13	14	8
Jul-25		9		15	15	16
Jul-18	Tarsus (mm)	9	10	10	12	8
Jul-20			10	11		9
Jul-22		11	11	11	11	10
Jul-25		11		11	12	11

Table 1. Data sheet (of box# 21) showing the measurements of nestlings during feeding experiment from July 18th to 25th 2004 (contd.)

Date		Spiked	Monroe	Point Pelee	Lake Winnipeg	Control	
Jul-18	Bill length (mm)	4	4	5	5	4	4
Jul-20		4	5	5		5	5
Jul-22		4	5	5	6	5	5
Jul-25		5		5.5	6	6	6
Jul-18	Ectoparasites	N	N	N	N	N	N
Jul-20		N	N	N	N	N	N
Jul-22		N	N	N	N	N	N
Jul-25		N	N	N	N	N	N

(0 = no fat visible, 1= some fat visible, 2=nearly filled with fat and 3= completely filled with fat) (Proctor and Lynch, 1993).

Blood samples were collected from the birds before necropsy examinations using a 25- gauge butterfly needle with a 2-3 mL syringe from the jugular vein into vacutainers containing lithium heparin 12.5 IU9 (Terumo Medical Corporation, Elkton, MD, USA). Samples were stored in cold until differential counts and other estimations were made in the Animal Health Laboratory, University of Guelph.

Ration determination

We determined the amount of food that could be supplemented to nestlings based on their estimated consumption rate. The consumption rate was calculated using bioenergetics principles and available published values (described in detail in chapter 4). The calculations to assess the amount of food supplemented to induce a specified bioaccumulation in the nestlings are included as Appendix 3 and Appendix Table 2).

Difficulties encountered with feeding experiments

The time required to feed 20 nestlings daily for 5-6 days was considerably more than anticipated, and we had only limited field assistance. Treating the mayflies with the carrier solvent, dichloromethane (applied to all treatments for consistency) tended to make the food hard and brittle, slowing the rate at which we could feed the nestlings. The small birds also required longer than expected to feed, and the parental interference (feeding) also reduced the amount of food that could be fed to the nestlings each day.

We couldn't perform contaminant analysis of the feed samples (mayflies) prior to the feeding experiments, so we fed the nestlings without knowing the actual contaminant concentrations of the various treatments.

Some of the nestlings were slightly older than our estimate or they showed a tendency to fledge early (which happens when humans handle the nestlings for experimental procedures). Consequently, we were forced to do necropsy procedures before our estimated time requirements. There were a few unexpected deaths of the nestlings during the feeding trials, but all carcasses were collected. Whenever necropsy

examinations were done, all tissues were collected, weighed and were stored frozen until analyses were performed.

Necropsy and other procedures

The nestlings were collected at approximately 11 days of age. They were weighed, and all relevant measurements and their migratory fat content were recorded (Table 2). The blood samples were taken and processed. The blood smears were made immediately by the slide to slide method. The nestlings were euthanized with an inhalation chemical (Halothane®; Halocarbon Laboratories, River Edge, NJ, USA), and examinations of the internal organs and collections of the internal organs were made. The internal organs were weighed and collected in 1.5 mL microtubes and stored for further examination if needed. The rest of the carcasses were skinned (the feathers were also collected) and were wrapped in xylene-rinsed aluminum foil. All samples collected were stored frozen at -20°C until the specified analysis were done.

Contaminant analysis

Contaminant analysis of the egg (Chapter 2), mayfly samples, the natural food of wren nestlings (Chapters 3 and 4) and bird tissues were done following standard protocol. Detailed description of the analysis protocol followed is given in O'Rourke *et al.* (2004) and Drouillard *et al.* (2007). The bird tissues were homogenised in a small, hexane-rinsed stainless steel blender. Additional drying and grinding were done in hexane-rinsed mortar and pestle mixing with sodium sulphate (VWR, Mississauga, ON). Moisture was determined for bird tissues. One g of each sample was dried in an aluminum weighing boat in an oven at 110 °C for 24 h and the dry mass was determined. Moisture content was determined from the change in weight between weighing.

Because the mayflies had already dried during the DCM treatment prior to the feeding experiments they could be homogenised very easily. Contaminants and lipids were extracted with columns of 25 g sodium sulphate and 50:50 hexane: DCM mixture (Fisher Scientific, Ottawa, ON).

Lipid contents of all the samples were determined following the method of Drouillard *et al.*, (2004). The liquid collected from each sample after sodium sulphate

Table 2. Data sheet showing the collection of organs and weight (g) and recording of other parameters of nestlings from box# 21 on July 25, 2005 after necropsy procedures (*Monroe mayfly fed was found dead before the completion of the experiment)

Organ	Spiked	Monroe*	Point Pelee	Winnipeg	Control
Breast muscle	0.58	N	0.64	1.18	0.9
Heart	0.07	N	0.06	0.1	0.1
Liver	0.34	N	0.34	0.43	0.43
Gall Bladder	0.02	N	0.03	0.01	0.01
Kidney	0.15	N	0.15	0.18	0.15
Thyroid	Y	N	Y	Y	Y
Thymus	N	N	Y	N	N
B.F	Y	N	Y	Y	Y
Spleen	Y	N	Y	Y	Y
Migratory fat	-1	N	-1	1	1
Excreta	Y	Y	Y	Y	Y
Blood Smear	2	N	2	2	2
Blood sample	Y	N	Y	Y	Y
Feathers	Y	Y	Y	Y	Y

extraction was rotoevaporated to 10 mL, and 1 mL of this was used for lipid determination. The 1-mL solute was dried in an aluminum boat for 1 h in a drying oven at 110 °C. The percentage of lipid in each sample was determined from this. Excess lipids in the samples were removed by gel permeation chromatography (GPC). The solid used in GPC column was porous SX-3 biobeads (BioRad, Mississauga, ON) and the solvent used for separating lipids and organochlorines was 1:1 DCM: hexane. All samples were cleaned and different contaminants were separated by using Florisil (VWR, Mississauga, ON) chromatography. Florisil (8 g of 60-100 mesh Florisil wet packed in hexane) and a topping of sodium sulphate were used as the column solid and different combinations of hexane and dichloromethane were poured sequentially through the column to separate different PCB congeners and other organochlorines. The solvents used were 50 mL of hexane (which separated PCBs (including mono-ortho substituted congeners), chlorobenzenes, octachlorostyrene, trans-nonachlor, pp'-DDE, mirex), 50 mL of 15:85 DCM:hexane (separating chlordanes, hexachlorocyclohexanes, cis-nonachlor, pp'-DDD and -pp'-DDT) and 130 mL of 60:40 DCM:hexane (which separated heptachloroepoxide and dieldrin).

PCBs and organochlorine analyses were completed by running 2 µL of the 1 mL rotoevaporated sample in a Hewlett-Packard 5890 gas chromatograph equipped with a 5973 mass selective detector (MSD) and a 5973 autosampler with a DB -5 column (Chromatographic Specialties, Brockville, ON, Canada). The sample was injected by splitless mode to a column with high purity helium which was used as the carrier gas in the machine. The mass spectrum obtained after machine run was analysed using the MSDChem software. The detection of individual chemicals was based on their retention time and major ion. The area obtained for each compound was compared against a standard area of a working standard diluted from a certified standard (Accustandard Quebec Ministry of Environment PCB Congener Mixture and Accustandard Custom Organochlorine Pesticide mixtures).

In order to ensure the quality of the procedure and to check the accuracy of the contaminant determination process, with every set (5 samples) a homogenate (an in house reference standard consisting of homogenised tissue of a large Common Carp from the

Detroit River with uniform chemical composition) sample and a blank sample were done. All samples were spiked with a surrogate standard (200 ng/mL of a mixture of C¹³ labelled PCB 37, PCB 52 and PCB153) to determine percentage recovery. Tissue contaminant concentrations obtained from the analysis were not corrected to surrogate standard recoveries as the recovery rates were within the acceptable range (65-135%). The (mean \pm SD; n= 5) concentration of PCB congeners 52 (86.55 \pm 55.23), 101 (182.21 \pm 51.73), 153 (372.67 \pm 101.2), 138 (327.53 \pm 34.13) and 180 (260.08 \pm 72.18) ng/g wet weight of in house reference homogenate were within the standard values given in laboratory control chart (mean \pm 3 standard deviation).

Data analysis

PCB congeners and other Σ DDTs were expressed on a wet weight (ww) basis. Different analyses were conducted using Statistica[®] version 6.1 software (Statsoft, Inc., 1997). One-way ANOVA was used to determine the significance of differences in concentrations of Σ PCB and Σ DDTs in the mayflies analysed, the initial weight of the nestlings and Σ DDT in different feeding groups. The differences between moisture content and % lipid content in the tissues of different feeding groups and feed samples were also determined by one-way ANOVA. Fisher LSD (least square difference) method was used for pairwise comparisons among means of the above groups. The differences in Σ PCB and relative growth rate after feeding were compared using randomized block ANOVAs, with nests treated as blocks and mayfly sources designated as fixed treatments. Planned comparisons were performed to see if there are any differences in the accumulation of PCB, differences in relative growth rate and other parameters among feeding treatments. Values were statistically adjusted to account for differences among nests. We predicted a gradient in the parameters in different feeding groups started with spiked (red)>Monroe (yellow)> Point Pelee (green)>Winnipeg (blue)> controls (white). As the variances among groups were not homogeneous, all data sets were log₁₀ transformed before statistical analysis.

Linear regressions were performed to assess the relationship between Σ PCB and individual congeners - orthoplanar congeners 118 – TEF= 0.00001; 105- TEF =0.0001; 156- TEF= 0.0001 and diorthoplanar congener 180- TEF =0.00001 summed TEQ of the

four congeners - (independent variables), and relative growth rate (dependent variable) of nestlings. The same analyses were used to estimate nestlings' elimination/retention/bio-transformation ability for different PCB congeners from readily cleared and slowly cleared congener groups based on their K_{ow} values. We performed regression analysis to assess the relationship between predicted versus observed values of PCB congeners belonging to readily cleared and slowly cleared congeners (grouped into one variable), and tested for correlation and also the homogeneity of slopes of different group regressions. This was to test the predictability of the model (Chapter 4). To see if there was any meaningful difference in different morphological variables measured and somatic index of internal organs weighed among different feeding groups, we used principal component analysis (PCA) to reduce the set of measurements to a smaller number of statistically independent factors. We then performed ANOVA on the PC scores. The significance was set at $p < 0.05$.

We analysed and quantified 38 PCB congeners (IUPAC #: 18, 17, 31/28, 33, 52, 49, 44, 74, 70, 95, 101, 99, 87, 110, 82, 118, 105, 151, 149, 153/132, 138, 158, 128, 156, 187, 183, 177, 171, 180, 191, 170, 201, 195, 194, 205, 208, 206 and 209). Σ PCB was determined as the sum of these 38 congeners analysed in various samples. We estimated Σ DDT as the sum of p,p'-DDT, p,p'-DDE and p,p'-DDD.

The PCB congeners and other organochlorines were classified into three groups according to Clark *et al.*, (1987) and Drouillard *et al.*, (2001; 2007) based on their persistence. Persistence of PCB congeners is mainly determined by the number of open *meta-para* positions on their biphenyl ring and their half life in different organisms. The classification of PCB congeners are as follows:

Rapidly cleared chemicals, with half lives of 10-60 d; OCS, dieldrin, PCB 31/28, 52, 49, 44, 70, 95, 101, 87, 110, 151, 149.

Intermediately cleared, with half lives >60-150 d: HCB, PCB 74.

Slowly cleared chemicals, with half lives > 150 d: oxychlordane, mirex, DDE, PCB 99, 105, 118, 153/132, 138, 158, 128, 156, 171, 177, 178, 180, 170, 183, 187/182, 195, 194, 201, 205, 206, 208, 209. The retention rates of the congeners were compared between the readily cleared and slowly cleared congeners.

Results

Contaminant burdens

The Σ PCB content in different mayfly groups used for feeding and also the Σ PCB content in the natural food of nestlings were compared and there were significant differences among them (ANOVA, $F(4,9)=200.1$, $p<0.0001$) (spiked vs. Monroe, $p<0.0001$; Fisher LSD; spiked vs. Point Pelee, $p<0.0001$; Fisher LSD; spiked vs. Winnipeg, $p<0.0001$; Fisher LSD; spiked vs. nestlings natural food, $p<0.0001$; Fisher LSD; Monroe vs. Point Pelee, $p<0.002$; Fisher LSD; Monroe vs. Winnipeg, $p<0.0001$; Fisher LSD; Monroe vs. natural nestling food, $p<0.0001$; Fisher LSD; Point Pelee vs. Winnipeg, $p<0.001$; Fisher LSD; Point Pelee vs. natural food, $p<0.0001$; Fisher LSD and mayfly from Winnipeg vs. natural food, $p<0.05$; Fisher LSD). They were in the expected order of spiked>Monroe>Point Pelee>Winnipeg and natural food (Table 3).

The lipid content (percent) of different mayfly groups used for feeding wren nestlings were compared using a one-way ANOVA. There was a significant overall difference among groups (ANOVA, $F(3,11)=8.01$, $p<0.01$). Spiked (18.51 ± 1.38 ($n=3$)) vs. Monroe (11.56 ± 1.38 ($n=3$)), $p<0.01$; Fisher LSD; spiked vs. Point Pelee (13.02 ± 1.07 ($n=5$)), $p<0.01$; Fisher LSD; spiked vs. Lake Winnipeg (18.37 ± 1.19 ($n=4$)), $p>0.05$; Fisher LSD; Monroe vs. Point Pelee, $p>0.05$; Fisher LSD. All these mayfly groups were significantly higher in lipid content compared to natural food of wren nestlings (4.55 ± 0.34 ($n=3$)), ANOVA $F(4,13)=21.74$, $p<0.001$ (Figure 1).

The moisture content in the tissues of different nestling groups (ANOVA, $F(4,19)=0.901$, $p>0.05$) and % lipid (ANOVA, $F(4,19)=0.23$, $p>0.05$) after feeding were not significantly different in different groups (Table 4). There were significant differences in the amount of Σ PCB accumulated by nestlings of different feeding groups (Randomized Block ANOVA, $F(3,15)=9.77$, $p=0.001$) (Table 4). Planned comparisons showed that each group was significantly different from others. The unmanipulated control contained significantly less Σ PCB than the other groups (control vs spiked, Monroe, Point Pelee and Winnipeg mayflies fed groups ($F(1,19)=42.12$, $p<0.0001$)). The Winnipeg mayfly fed group was different from spiked, Monroe and Point Pelee mayflies fed ($F(1,19)=54.28$, $p<0.0001$) and also spiked mayfly fed group was different from

Monroe and Point Pelee mayfly fed ($F=(1,19)=171.77$, $p<0.0001$) and also Monroe mayfly fed group was significantly different from Point Pelee mayfly fed groups ($F(1,19)=6.73$, $p<0.05$). These results showed that that spiked mayfly fed group had higher Σ PCB than others followed by Monroe mayfly fed group followed by Point Pelee mayfly fed, followed by the Winnipeg mayfly fed birds and the lowest concentration was exhibited by the control group (Table 4). When mean DDE accumulated between groups were tested for differences, there were no significant differences among groups (ANOVA, $F(4,19)=1.506$, $p=0.20$) (Table 4).

Growth and other morphological markers

We didn't find any ectoparasites on the nestlings, in the nest cups or in the nestling materials of any of the nest boxes. Migratory fat deposits were lower (visual) in spiked mayflies and Monroe mayflies groups but were similar in the other three groups. There was no initial difference in the weights of nestlings that were assigned to different feeding groups (ANOVA, $F(4,20)=0.02$, $p>0.05$) (Figure 2). Nestlings' final weights at the end of feeding experiments were significantly different among groups (ANOVA, $F(4,20)=7.01$, $p<0.001$) (red vs. yellow, $p>0.12$; Fisher LSD; red vs. green, $p>0.62$; Fisher LSD; red vs. blue, $p<0.03$; Fisher LSD; red vs. control, $p<0.004$; Fisher LSD; yellow vs. green, $p<0.04$; Fisher LSD; yellow vs. blue, $p<0.001$; Fisher LSD; yellow vs. control, $p<0.0001$; Fisher LSD; green vs. blue, $p>0.09$; Fisher LSD; green vs. control, $p<0.01$; Fisher LSD and blue vs. control, $p>0.35$; Fisher LSD (Figure 2) (Table 4). When the relative growth rate (RGR), *i.e.*, growth rate relative to the initial weight was calculated ($(\ln W_{t_2} - \ln W_{t_1})/(t_2 - t_1)$ where W_{t_2} was the weight before feeding experiments and W_{t_2} weight after the experiments) (Brown *et al.*, 1988) and compared among groups there were significant differences among groups after feeding with the mayflies (Randomized Block ANOVA, $F(3,15)=9.77$, $p=0.001$) (Figure 3). Planned comparisons showed that the control group was significantly different from others with highest relative growth rate (control vs. other groups ($F(1,20)=7.4$, $p<0.01$)). Monroe mayfly fed group showed the lowest growth and was significantly different from others (Monroe vs. others ($F(1,20)=16.15$, $p<0.001$)). Spiked mayfly fed groups showed growth rates significantly lower than the Winnipeg mayfly fed (spiked vs. Winnipeg group ($F(1,20)=5.13$, $p<0.05$)).

but showed no difference to the group that was fed with mayflies collected from Point Pelee (spiked vs. Point Pelee ($F(1,20)=0.66$, $p>0.05$)). Nestlings that were fed with mayflies collected from Point Pelee National Park area and Lake Winnipeg were growing at the same rate (Point Pelee vs. Lake Winnipeg fed groups ($F(1,20)=3.32$, $p>0.05$)) (Figure 3).

Principal component analysis showed that the first factor was positively associated with external measurements such as primary feather length, wing chord length, tail length, tarsus length and bill length (the measured lengths-to-body-weight ratio (Hoffman *et al.*, 1993), the second factor was associated positively with liver (Liver Somatic Index, LSI) and kidney (KSI) weights and the third factor was positively associated with heart and breast muscle weight. All weights were calculated as somatic indices). These three factors together explained 78% variation (Table 5). We did a one-way ANOVA on factor scores and found that there were no significant differences in the values of factors one or three among feeding groups, but there was a significant difference in values of factor 2 scores among feeding groups. Monroe mayfly-fed nestlings showed positive association with high liver and kidney weight proportional to their final body weight (ANOVA Factor 1 and feeding groups $F(4,19)=1.65$, $p>0.05$; Factor 2 and feeding groups $F(4,19)=18.39$, $p<0.0001$ and Factor 3 and feeding groups $F(4,19)=1.83$, $p>0.05$). The Monroe mayfly-fed group was significantly different from others (Monroe vs. spiked $p<0.001$; Fisher LSD; Monroe vs. Pelee $p<0.0001$; Fisher LSD; Monroe vs. Winnipeg $p<0.0001$ and Monroe vs. control $p<0.001$; Fisher LSD; spikes vs. Pelee $p>0.05$; Fisher LSD; spiked vs. Winnipeg $p<0.01$; Fisher LSD; spiked vs. control $p<0.01$; Fisher LSD; Pelee vs. Winnipeg $p>0.05$; Fisher LSD; Pelee vs. control $p>0.05$ and Winnipeg vs. control $p<0.05$) (Figure 4).

We performed regression analyses to assess the relationship between RGR and Σ PCB as well as with 4 PCB congeners with known toxic equivalency factors (TEF) for birds (mono-orthoplanar congeners 118 – TEF= 0.00001; 105- TEF =0.0001; 156- TEF= 0.0001 and diorthoplanar congener 180- TEF =0.00001) there were no significant correlations. (Σ PCB against RGR: $r=-0.34$, $p=0.1$ and RGR and congeners 118, 105, 156 and 180 (Figure 5). But when the group fed on spiked mayflies were removed from the

Table 3. Mean \pm SE as indicated (ng/g) Σ PCB and DDE in mayflies used for feeding and the Σ PCB expected to accumulate and Σ PCB observed in different feeding groups.

Feeding group	PCB mayflies	PCB nestling tissue	DDE ng/g	PCB predicted	% Σ PCB retained
Spiked	35870.8 \pm 1142.3(n=3)	21970.5 \pm 13907.6 (n=4)	82.1 \pm 55.6 (n=4)	20117.17	109.21
Monroe	893.9 \pm 91.1 (n=3)	883.9 \pm 273.8 (n=5)	114.2 \pm 38.9 (n=5)	1240.40	71.26
Point Pelee	284.9 \pm 33 (n=3)	421.7 \pm 30. (n=5)	104.2 \pm 80.9 (n=5)	660.93	63.76
Lake Winnipeg	55.2 \pm 16.2 (n=2)	350.4 \pm 28.1 (n=5)	56.9 \pm 66 (n=5)	552.16	63.46
Controls		300.5 \pm 44. (n=5)	111.7 \pm 36. (n=5)	514.33	58.42

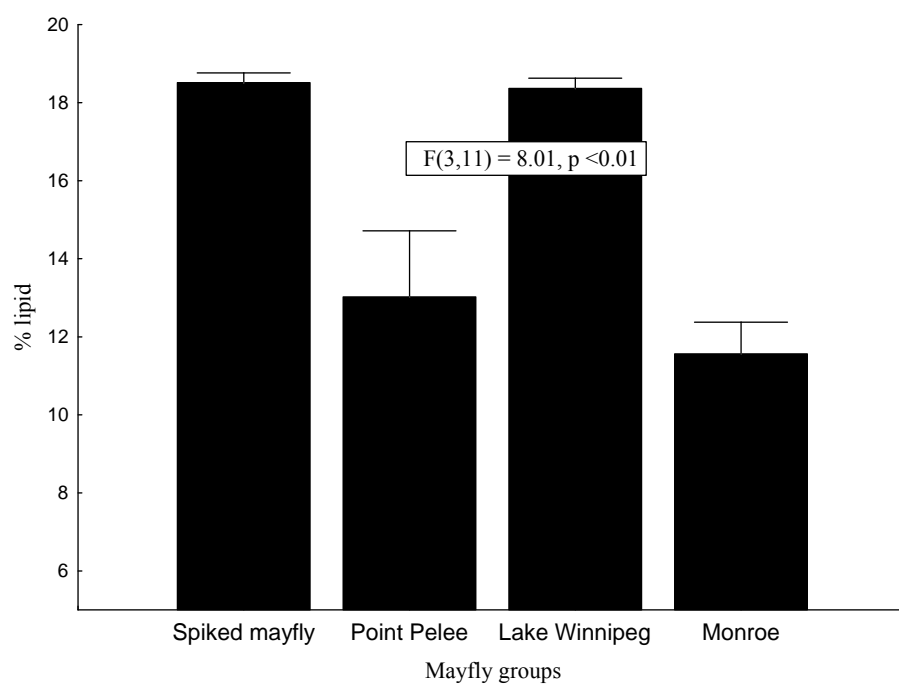


Figure 1. Mean \pm SE % lipid in mayflies from different locations (spiked $n=3$, Monroe $n=3$, Point Pelee $n=5$ and Lake Winnipeg $n=4$)

Table 4. Different feeding groups, amount of mayflies fed and different parameters measured in them

Box # and feed type	Amount fed (g)	% lipid	% moisture	Σ PCB ng/g	DDE ng/g	Initial weight (g)	Final weight (g)
Spiked 11	5	4.53	31.3	44621.4	82.2515	7.1	7.7
1	4.5	4	27.04	20686.1	164.433	7	8.8
21	5	2.23	25.4	15205.7	73.735	9.1	8.2
15	4	6.48	29.22	7368.68	7.82041	9.5	8.5
4						10.7	8.2
Monroe11	5	2.97	27.29	1281.48	71.3353	7.9	6.9
1	4.5	4.63	30.89	867.824	187.292	6.2	5.9
21	5	2.27	27.79	1075.93	98.2244	8.6	7.8
15	4	6.96	34.05	510.45	102.88	10.1	7.6
4	4	2.86	24.53	683.687	111.044	11.6	8.78
Point Pelee 11	5	5.83	32.73	386.281	87.6303	7.8	8.9
1	4.5	6.56	30.32	464.372	254.929	8.1	10.2
21	5	2.17	26.38	435.485	73.7972	10	9.2
15	4	4.09	31.15	388.176	10.7474	8.1	8.5
4	4	1.89	25.94	432.66	93.96	10.7	6.5
Winnipeg 11	5	6.51	31.33	319.974	25.0896	7.6	10
1	4.5	4.46	30.81	330.254	6.44279	7.7	8.2
21	5	4	28.3	365.407	160.931	10.3	10.5
15	4	3.22	31.1	338.638	7.45727	9.9	10
4	4	5.62	25.98	397.758	84.5694	8.7	10.2
Control 11		7.22	31.46	248.721	146.984	7.2	10.8
1		5.12	26.78	311.938	155.055	9	11
21		5.09	28.54	310.8	112.444	8.4	9.07
15		6.41	25.65	259.267	61.6942	9.8	10.6
4		4.19	28.2	371.691	82.3807	9.8	10.8

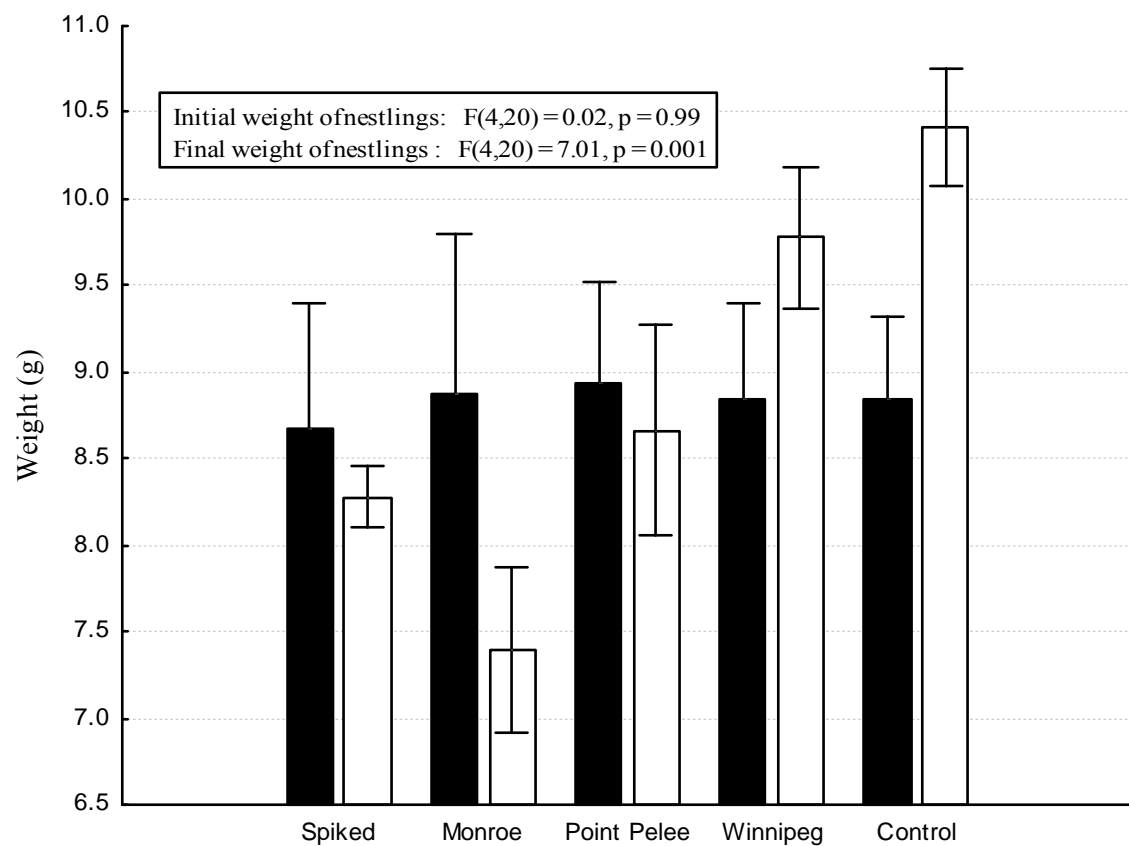


Figure 2. Mean \pm SE nestling biomass (g; n=5 in each group) before (5 days old) and after (9 days old) feeding mayflies of different PCB concentrations and control

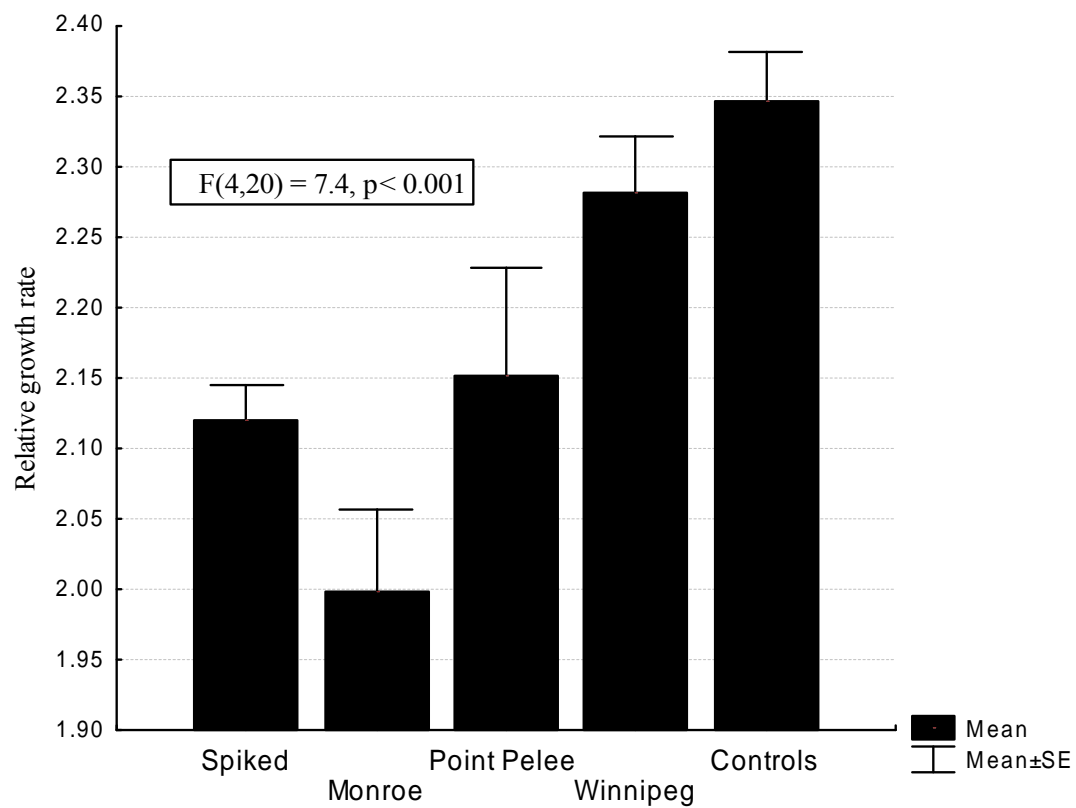


Figure 3. Mean \pm SE (n=5 in each group) relative growth rate of nestling over 5 days of feeding with mayflies of different PCB concentrations and control

analysis, all showed significant correlations (Σ PCB against RGR: $r=-0.69$, $p=0.001$ and RGR and congeners 118, 105, 156 and 180 (Figure 5). When regression analysis were conducted with combined TEQ of the four PCB congeners against RGR (without spiked group) showed significant negative regression (TEQ against RGR: $r=-0.61$, $p<0.01$) (Figure 6).

Hematological variables

We estimated the total white blood corpuscle (WBC) count, the differential count of lymphocyte, monocyte, basophil, eosinophil and hetrophil, and also the ratio of hetrophils to lymphocytes. Even though there were some trends that showed contaminant levels in the nestlings, there were no significant differences in all of the parameters measured. There was no significant difference between total WBC among groups (ANOVA, $F=(4,7)=2.01$, $p=0.19$; spiked $n=2$; Monroe $n=2$; Point Pelee $n=3$; Lake Winnipeg $n=2$; and control $n=3$) (Figure 7). There were no significant differences in polychromasia (related to maturation of erythrocytes) and in hematocrit of the nestlings.

Application of bioenergetics model

When all groups were tested for goodness of fit between predicted and observed values, overall they showed significantly positive slopes, except PCB spiked mayfly fed groups ($t=5.41$, $p<0.001$) (Figure 8). The homogeneity of slope analysis showed that the elevations of the curves did not differ significantly ($t=0.06$, $p>0.05$). Regression analysis of readily cleared congeners in nestling tissues belonging to different feeding groups against K_{ow} showed a positive relationships, with most slopes significantly greater than zero (spiked, $r=0.31$, $p=0.04$ Monroe, $r=0.5$, $p=0.03$; Point Pelee, $r=0.55$, $p=0.03$; Lake Winnipeg, $r=0.63$, $p=0.005$ and control, $r=0.53$, $p=0.03$) (Figures 9 and 10). Regression analysis with slowly cleared congeners observed values in nestling tissues in different feeding groups against K_{ow} showed negative non significant correlations (all groups expect spiked $n=5$ and spiked $n=4$, spiked, $r=-0.38$, $p=0.19$; Monroe, $r=-0.12$, $p=0.12$; Point Pelee, $r=-0.27$, $p=0.22$; Lake Winnipeg, $r=-0.3$, $p=0.18$ and control, $r=-0.24$, $p=0.28$) (Figures 10 and 11).

Table 5. Factor loadings of morphological variables and somatic index of internal organs
(Extraction: Principal components)

Variable	Factor 1	Factor 2	Factor 3
Tarsus length	0.89	-0.05	0
Bill length	0.88	0.19	0.03
Tail length	0.86	-0.2	0.19
Pr. Feather length	0.85	0.15	0.01
Wing chord length	0.8	0.01	-0.13
Liver weight	0.18	0.91	0.24
Kidney weight	-0.21	0.86	-0.14
Heart weight	0.11	0.13	0.93
Breast Muscle	-0.3	-0.47	0.55
Expl.Var	3.85	1.91	1.3
Prp.Totl	0.43	0.21	0.14
Cum Prp	0.43	0.64	0.78

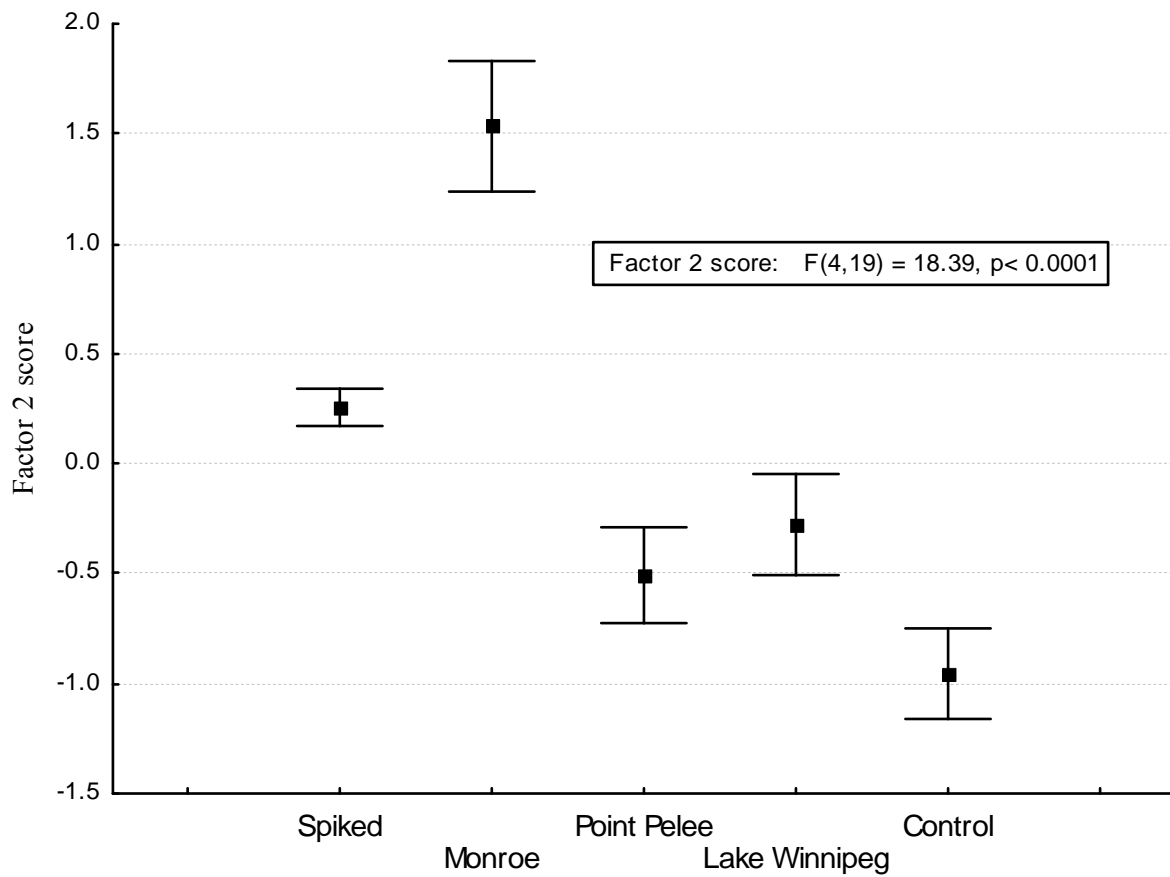


Figure 4. Relationship between factor score 2 and different feeding groups

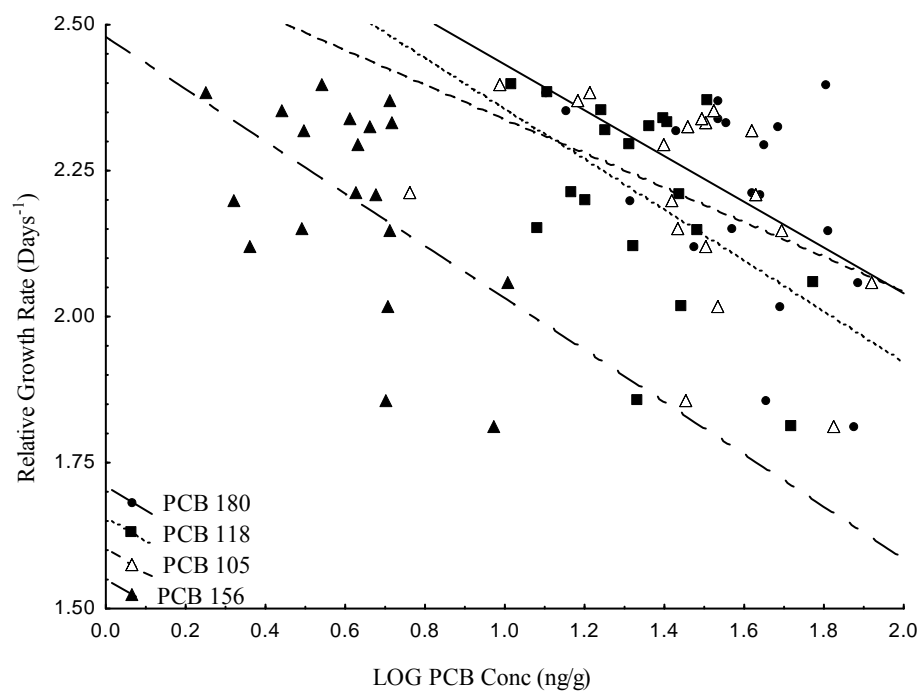


Figure 5. Relationship between relative growth rates of nestlings and PCB congeners with known TEF values (nestlings fed on spiked mayflies are excluded)

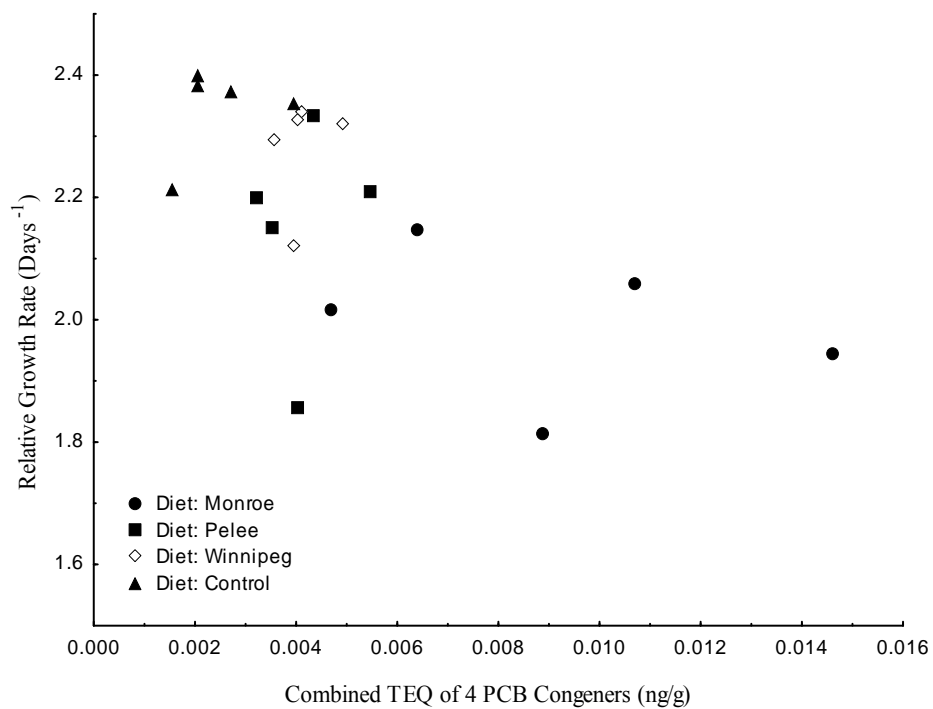


Figure 6. Relationship between relative growth rates of nestlings and combined TEQ of 4 PCB congeners (nestlings fed on spiked mayflies are excluded)

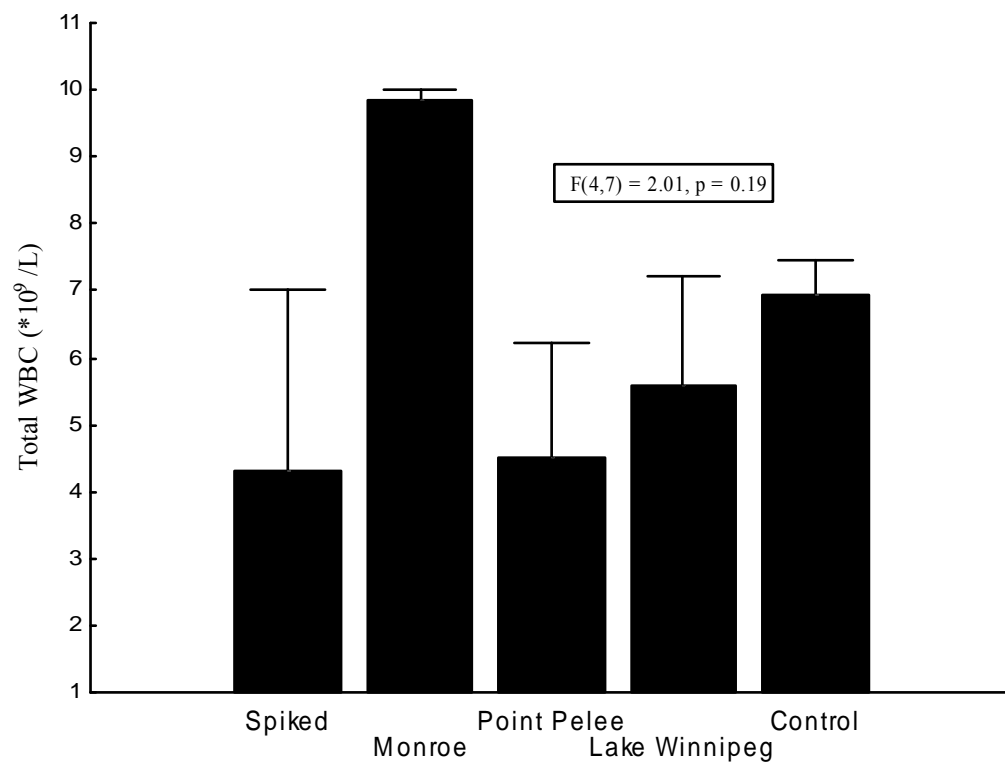


Figure 7. Mmean±SE total white blood cell counts in wren nestlings (spiked, n=2; Monroe, n=2; Point Pelee, n=3; Lake Winnipeg, n=2 and control, n=3) after feeding with mayflies of different PCB concentrations

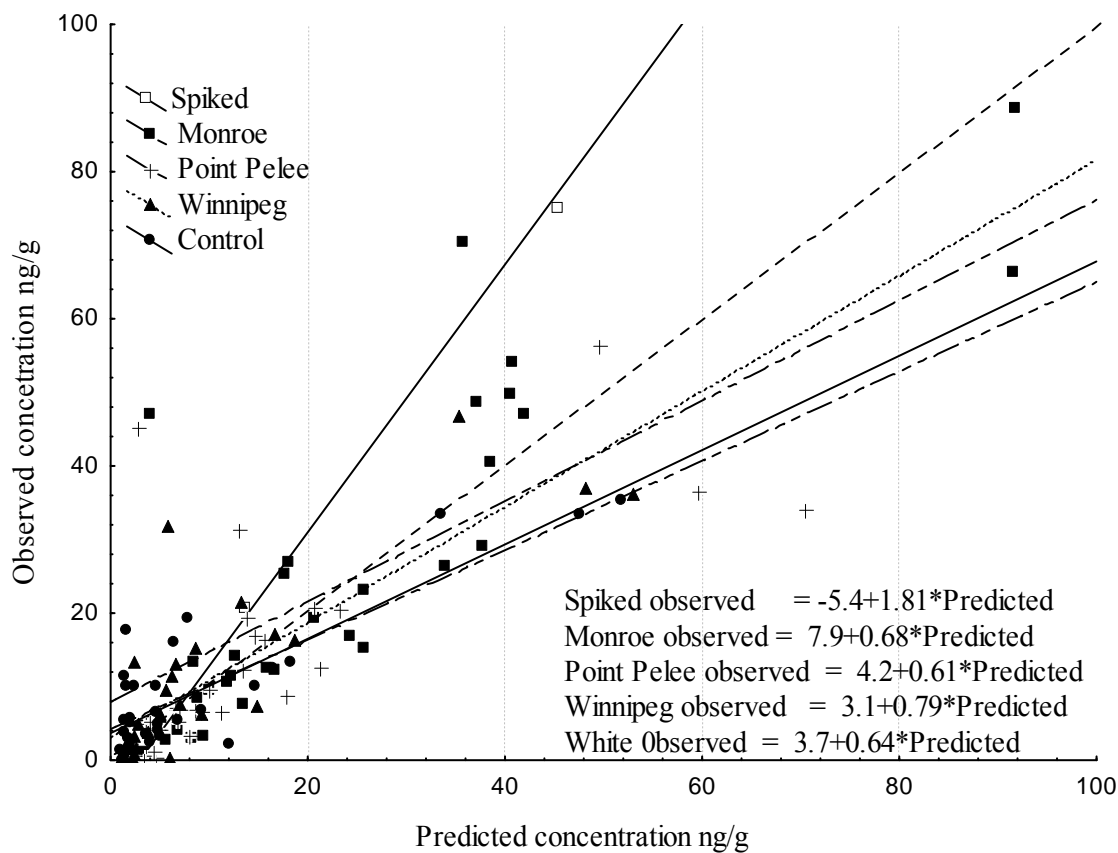


Figure 8. Regression of observed vs predicted concentrations in ng/g for PCB congeners belonging to different feeding groups

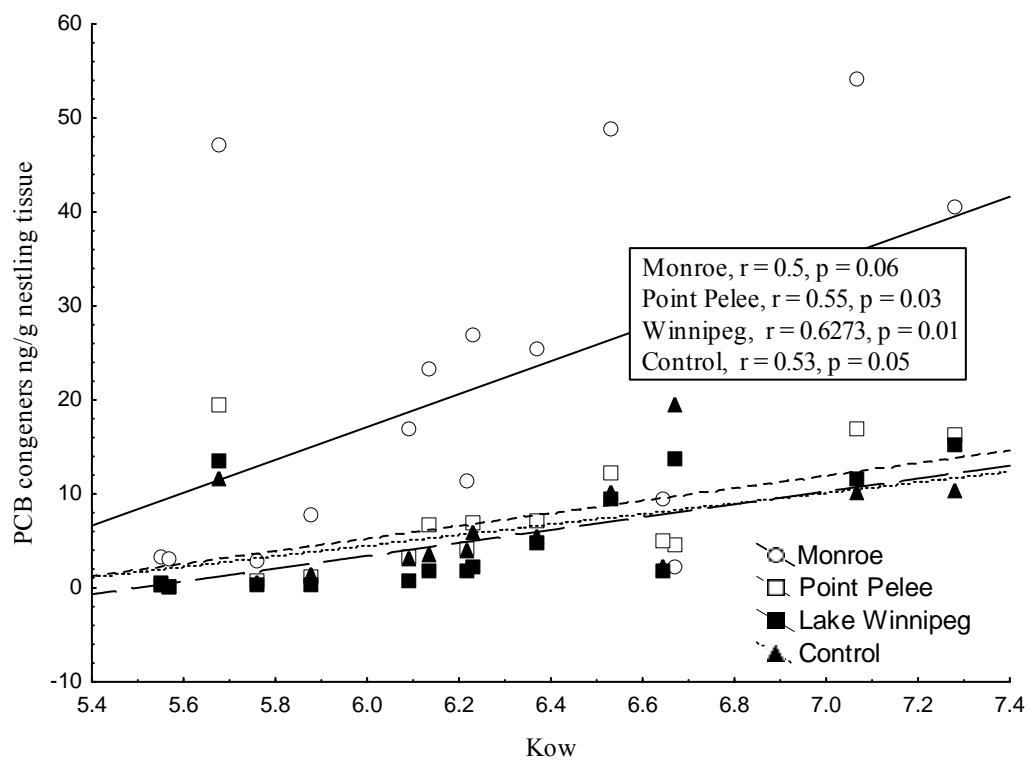


Figure 9. K_{ow} vs readily cleared PCB congener concentration in different feeding groups (Monroe $n=5$; Point Pelee $n=5$; Lake Winnipeg, $n=5$; and control $n=5$)

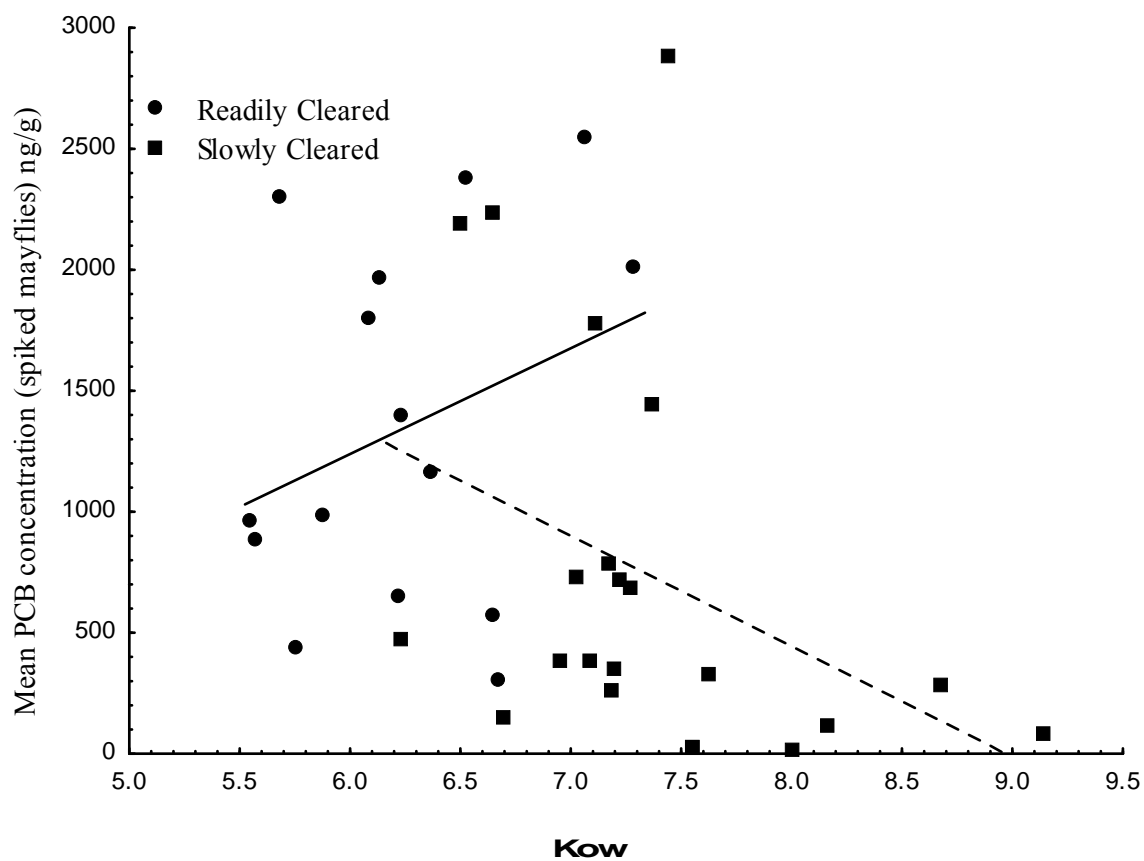


Figure 10. Concentrations of PCB congeners vs K_{ow} for nestlings fed with spiked mayflies ($n=4$) (—) regression readily cleared congeners ($r=0.31$, $p>0.05$) (---) slowly cleared congeners ($r=-0.38$, $p>0.05$)

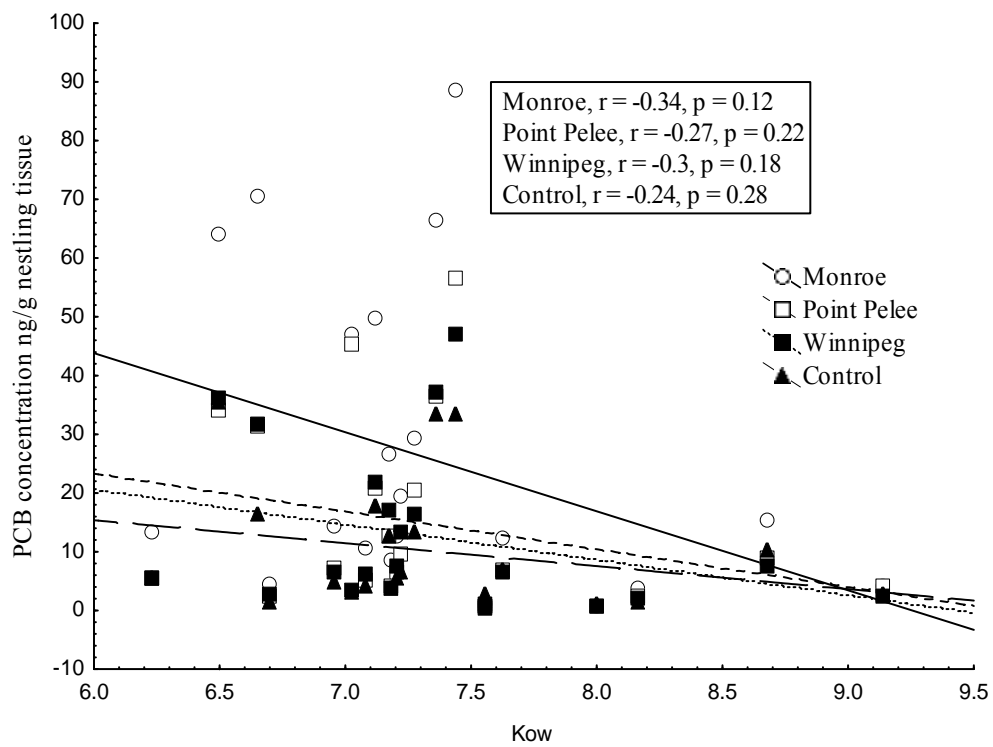


Figure 11. K_{ow} vs slowly cleared PCB congener concentration in different feeding groups (Monroe $n=5$; Point Pelee $n=5$; Lake Winnipeg, $n=5$; and control $n=5$)

Discussion

The bioaccumulation of PCBs in nestling tissues was consistent with the concentrations expected in the nestlings fed with different supplements. Nestlings showed the greatest level of accumulation when fed the spiked mayflies. This accumulation pattern was in agreement with the level of Σ PCB in different groups of mayflies used for feeding trials. The natural food of wren nestlings had levels of Σ PCB that were much lower than the foods we introduced to them through feeding trials. The depletion of PCB in the natural food of wren nestlings was expected as the major insects found in their diet were mainly terrestrial insects and was reflective of wrens' feeding strategy (gleaning in the bushes). The nestlings' diets were mainly composed of grasshoppers, spiders and adult moths and their caterpillars (chapter 3). Since the soil of Point Pelee National Park is less polluted with PCB than are the sediments of adjacent Lake Erie, the accumulation from terrestrial habitat by terrestrial insects is expected to be less than from insects like mayflies, which accumulate their contaminant load from Lake Erie sediments. Multiple sources have contaminated Lake Erie sediments with PCBs at different levels at different locations (Gewurtz and Diamond, 2003; Marvin *et al.*, 2004; Heidtke *et al.*, 2006).

The absence of ectoparasites on nestlings and surroundings was a good indication that the wren nestlings were healthy and were developing in healthy surroundings. Migratory fat, which is again an indication of health of nestlings showed a PCB related trend but as it is a visual measure it needs more strict quantification before incorporating it as pollution related impairment.

The nestlings' final body weight at the end of the study differed significantly as a function of the type of mayfly supplement. The spiked and Monroe, MI groups were significantly lighter than all of the other groups. The Monroe group was significantly different from all groups except spiked mayfly fed group. The spiked group and Point Pelee group were the same in final weight were different from manipulated control (Winnipeg mayfly fed group) and from unmanipulated control group. Final weight of the Monroe group was the most noticeably different from all other groups. This was evident when the relative growth rates of different groups were compared. The other most abundant organochlorine, DDT showed no significant difference among groups. So it is

not clear whether the observed difference in growth is because of the organic contaminants examined in this study or due to some other contaminant, such as metals that may be present especially in the Monroe group (mayflies collected from Monroe, MI). In the wild, the additive (effects of two contaminant added up), the synergistic (when combined effects of two contaminants are more than added effects of two individual contaminants) and potentiation (one chemical with no harmful effect becomes contaminant with effects in the presence of another contaminant) effects of other contaminants and physical characteristics are important in showing some effects in targeted species. Sterling State Park (Monroe) from where the mayflies were collected is near River Raisin, which was designated an Area of Concern (AOC) by United States and Canada in 1987 due to the heavy contamination of that site by oils and grease, heavy metals and PCB (Corkum *et al.*, 1997).

Comparison of the PCA-derived morphometric scores among feeding groups showed that the Monroe mayfly fed group had higher LSI and KSI, findings that are consistent with other studies. Hoffman *et al.*, (1996) found liver enlargement (hepatomegaly) and coagulative necrosis with 156 ng/g wet weight of PCB 126 in posthatching kestrels. Ian Prestt, *et al.*, (1970) found enlarged kidneys in birds that died of PCB contamination in Britain. Papp *et al.*, (2005) found a positive correlation between PCB concentration and LSI in tree swallows raised in Point Pelee National Park. They suggested that the increased activity of detoxification enzymes was the reason for increased liver size. In this study the Monroe mayfly fed group, which showed low growth rate and lowest body weight after experimental period had enlarged livers and kidneys. This enlargement of detoxification and elimination organs may indicate the activation of detoxification machinery. This is in confirmation with Papp *et al.*, (2005). So overall the reduction in growth as well as increased liver size in spiked food fed nestlings above that of Winnipeg fed nestlings indicate that PCB do contribute to the noticed difference but some contaminant in Monroe mayflies was contributing a greater impact in those nestlings that fed on Monroe mayflies.

All nestlings in the study received organochlorine contaminants that were maternally transferred when yolk lipids were deposited for the formation of eggs (Drouillard, 2000). The maternal transfer of pollutants in most cases depends on the egg

sizes and amount of yolk lipid (Barron *et al.*, 1995) and in the present case there were nontrivial amounts transferred to nestling by their parent, as the eggs were contaminated with PCB congeners (Chapter 2). Female wrens could have transferred the contaminants they previously acquired on their wintering grounds during the time of their egg production (Bishop *et al.*, 1995) so that the contaminant burden in the egg may reflect maternal burden accumulated on wintering grounds (Martin *et al.*, 2003). We found comparatively higher level of PCBs categorized as persistent congeners in wren eggs than eggs of bird species that fed on aquatic insects (Chapter 2). The diet of wrens has more terrestrial insects (Skutch 1953; Neigh *et al.*, 2006; Chapter 3). Terrestrial insects in the park are depleted of PCBs compared to aquatic insects (Smits *et al.*, 2005; Chapter 3). The diet primarily of terrestrial origin which carry less PCB (compared to aquatic insects in Point Pelee National Park (Smits *et al.*, 2005)) and the abundance of highly persistent PCB congeners in the eggs of this species make it clear that they were deriving those PCB burden from overwintering habitat with unknown PCB concentrations. This was transferred to all nestlings in the same composition and concentration. So in this study we assumed that all the nestlings received comparable amounts of contaminants from their mothers and that all the nestlings received the same type and amount of contamination through the food provided by the parents. Randomized block ANOVA showed that there was no significant variation in RGR from nest to nest. Consequently, the additional PCB burdens evaluated in nestlings were acquired through the different food supplements (mayflies) that we provided during the experimental period and that led to the variations in RGR.

We found lipid content in Monroe mayflies was significantly lower than Lake Winnipeg mayflies but similar to those from Point Pelee. But the lipid content of the food was not affecting the growth as was evident from the similarity in growth of Point Pelee and Lake Winnipeg mayfly fed groups. Their relative growth rate and final body weight were not significantly different between Point Pelee group and Winnipeg mayfly fed group. The low fat content of natural food also points to the fact that low fat content of Monroe mayfly group was not a limiting factor for normal growth of the nestlings.

Another factor that could affect growth of nestlings was repeated handling. Parsons and Burger (1982) studied effects of handling on black-crowned night herons

nestlings measuring their weights after handlings. They found no significant difference due to handling compare with controls. However, nestlings that were handled more frequently grew more slowly than nestlings that were handled less frequently in cassin's auklets (Morbey and Ydenberg, 1997). Even though the growth rate was slower, handled nestlings reached greater peak weight than the control group. In the present study, the control nestlings showed normal growth (see Chapter 4) even though they were also captured for taking morphological measurements along with other experimental nestlings. So also the nestlings belonging to different feeding groups were growing at different rates.

We also assumed that any differences seen in nestling morphology or hematology variables among treatments would be due to additional PCB. The total WBC count was higher in the nestling group fed with Munroe MI mayflies (highly contaminated), even though there was no overall significant difference among the 5 study groups. We could collect only very few blood samples due to many difficulties encountered during our experiments. Increase in total WBC count is well documented as pollution-bound effect. Mandal *et al.*, (1986) studied hematological changes after oral administration of lindane (γ -hexachlorocyclohexane) to 6 species birds and found that total WBC counts were increased while the differential counts of WBC were mainly associated with increases in hetrophil and eosinophil counts; they noticed a decrease in monocyte, lymphocyte and basophil numbers. They suggested hematological changes induced by lindane could serve as an early warning signal for pesticide toxicity. In our study, the trend in increase in total WBC counts in nestlings with lowest growth rate and low body weight were possible indications of pesticide and PCB related toxicity.

Mayflies were collected from different locations make their tissue burdens inherently different as they were developed in different geographical area. This could be a reason for different growth rates. But the significant differences seen between nestlings fed spiked mayflies (which was PCB spiked mayflies collected from Lake Winnipeg area) and the nestlings fed by non spiked Lake Winnipeg mayflies (manipulated control) in their final weight are definitely due to the additional PCB provided to them through feeding. But the significant differences in final weight and RGR which was higher in

group that bioaccumulated PCB to the highest level, cast doubt on the PCB toxicity as the major factor affecting growth retardation.

The regression analysis showed significant correlation with Σ PCB, 4 PCB congeners whose TEF is known and total TEQ (ng/g) against RGR were all correlated significantly. Even though a linear fit was there with 4 PCB congeners of known TEQ (dependent variable) and RGR (Figure 5) and the relationship between TEQ of congeners (dependent variable) and RGR (Figure 6), there are different groups which are clustered based on feed types and this suggests that PCB concentration in each feed type is affecting growth rather than PCB concentration per se. Even though the natural mayfly fed groups were aligned on the linear regression line well, the separation of artificially PCB spiked mayfly group away from the main frame needs more exploration into the different contaminant burdens in different groups of mayflies and the real effect of PCB as a pollutant at least in this study species.

The feed ration for nestlings for each day and for different days (as the nestlings were growing) was developed based on the bioenergetics based model. So the observed bioaccumulation in nestlings' tissues could be used to validate the model. The plot of observed versus predicted Σ PCB (predicted based on the PCB contributed by the egg plus contribution from the food provided by the parents plus the food supplemented by feeding experiments) showed that the correlations were significant and the slopes were not significantly different (Figure 8). The predictions were made based on the consumption estimation made for wren nestlings from Chapter 4. Even though the predictions were different (from $0 + 1.0 \times$ predicted) in most cases with over prediction in all natural feeding groups and under prediction for spiked mayfly fed group, it was well within expected range for this kinds of models. The lower observed values for readily cleared congeners (with vicinal H-atom substitutions at *meta*-, *para*- carbons) could be attributed to biotransformation and elimination. The lower prediction of bioaccumulation of slowly cleared congeners (without vicinal H-atom substitution) could be attributed to low absorption rate for those congeners from gastrointestinal tract (Norstrom, 2002). In the nestlings that were fed with spiked Lake Winnipeg mayflies, both groups of congeners were under predicted. This was due to high variability in the samples of mayflies analysed, perhaps due to heterogeneous adsorption of PCB in the mayfly

samples during spiking process. If the predictions were made with the mayfly sample that exhibited the highest concentration, the regression coefficient aligned with other groups.

The regression analysis with PCB congeners from readily cleared congeners against the most important physicochemical property (K_{ow}), they showed positive correlation and most groups showed significant correlations. The slowly cleared congeners against K_{ow} showed a negative trend and none of the correlations was significant. This relationship was shown by two other species passerine nestlings (tree swallows and purple martins, when bioenergetics model was used to predict PCB accumulation of readily cleared congeners and slowly cleared congeners in them (Chapter 4). This commonality of retentions of readily cleared congeners shown by the nestlings of different feeding groups may be due to their having inadequate time to eliminate the PCB congeners absorbed from the intestine. Clark *et al.*, (1987) showed that experimental juvenile herring gulls showed longer PCB burden half life (slow elimination) compared to wild juveniles and an even slower rate than wild adults for most of the organochlorines they studied. In their study, for example, experimental juveniles had a DDD half life of 47.5 d wild juveniles had a half-life of 15 d wild adult female half-life was 9.8 d. They attributed the longer half lives in juveniles to their higher lipid content compared to adults. Our study, which lasted only a few days (7 d), allowed very little time for nestlings to metabolize and eliminate those persistent chemicals. Few studies have explored the biotransformation elimination in juvenile birds. Drouillard *et al.*, (2007) studied the elimination of different PCB congeners in juvenile American kestrels and observed that the juveniles and adults observed the same structural activity rules for PCB metabolism. But they also reported the half-life that was much longer than what we observed in the present study.

The underlying goal of this experiment was to evaluate the effectiveness of ways to identify nestling exposure to bioaccumulative pollutants in terms of morphological and haematological markers that can be measured without harming the nestlings. The experiment was conducted with rigorous attention to standard protocols. But for many reasons the results especially some of the biomarkers (haematological parameters) could not be assessed properly. So if an experiment is going to be conducted for wild

populations of birds in natural settings, it is advisable to have better method to spike natural food items, better ways to feed the nestlings, larger sample size etc.

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Chapter 6: Avian non-destructive biomarkers of organochlorine contamination- a review

Introduction

Persistent pollutants from the environment cause impairments in animals and plants and in severe cases produce irreversible damage to individuals, populations, communities and ecosystems. In most cases these irreparable changes begin in individuals as very minor changes (if the dosage of the pollutant is low) and then advance to a stage where recovery is not possible. In the early stages of exposure affected individuals show some subtle changes or signals and symptoms which, if diagnosed and assessed properly and treated appropriately (the cause) could prevent or minimize the effects of succeeding impairments. Treatments range from very simple remedial measures to complete clean-up of the source and sink of that particular pollutant. The early warning signals shown by individual entities in their biochemical or physiological conditions are called biological markers (biomarkers). Biomarkers can be indicators of exposure or can be indicators or predictors of impairment or effects of exposure (Forbes *et al.*, 2006). Proper detection and interpretation of these biomarkers is important to protect individuals and consequently populations, community and the ecosystem from the multitude of pollution-related problems. In other words, biomarkers are an essential tool in biological/environmental monitoring.

A biomarker is a quantifiable, time dependent or dose dependent biological response or toxic effect elicited by a xenobiotic substance at the individual level or below (Mayer *et al.*, 1992; Walker, 1998). It is a measure of exposure to a toxic substance (Timbrell, 1998), or of toxic effect at the histological, biochemical, physiological, morphological or behavioral level (Jamil, 2001). It integrates both time and space in its expression based on bioavailability (van Gestel and van Brummelen, 1996). Another definition is “a change induced by a contaminant in the biochemical or cellular component of a process, structure or function that can be measured in a biological system” (NRC, 1989). Because biomarkers are sought as diagnostic tools that are more easily measured than the underlying phenomenon, they should be quantifiable, sensitive, non-invasive, specific, and easy to measure; and they should relate to biochemical

mechanisms, and work at realistic doses of exposure to xenobiotics (Walker, 1998, Timbrell, 1998). Biomarkers become more relevant when the measured entities are essential for normal functioning of cells, tissues, or the organism (Handy *et al.*, 2003). It is advisable to have multiple biomarkers to characterize the impact of different concentrations of contaminants, adverse effects due to contaminants and to quantify toxic damage than it is to consider a single response (Handy *et al.*, 2003). Biomarkers can be used to track each phase of the dose–response continuum, from exposure through irreversible effect or to resultant disease (Fox, 1993; Schmidt, 2006).

Even though biomarkers are used extensively in ecotoxicology, sometimes the associated language is used vaguely, and can be confused with terms that are more properly applied to the general study of biological indicators or ecological indicators. Redefinitions of the following three terms are provided by van Gestel and van Brummelen (2006) who differentiated biomarkers from biological indicators and ecological indicators. According to them, all measurements of health in terms of an organism's biochemical, physiological, histological and morphological characteristics are termed biomarkers. This excludes the behavioural effects. Behavioural effects along with other information such as an organism's presence or absence in a habitat due to certain environmental conditions prevalent in that particular habitat, are classified as biological indicators by the authors. Ecological indicators are described as measures of changes in ecological structure and functions above the level of the individual, such as species diversity, population dynamics, community structure, nutrient cycling rates etc. In this paper the main focus is biomarkers. However, selected biological indicators are also discussed.

There are many analytical methods available to detect and quantify the amount of toxins or their breakdown products in different animal species. But the accurate measurement of these chemicals depends on a thorough understanding of their chemistry and their actions. According to Walker (1998), the four most desirable characteristics for biomarker assays are sensitivity, specificity, simplicity and stability.

Timbrell (1998) divided biomarkers into following three groups

1. biomarkers of exposure;
2. biomarkers of response or toxic effect;
3. biomarkers of susceptibility. (Fig. 1)

Biomarkers of exposure are important in toxicology, because they are indicators of internal dose, or the amount of chemicals absorbed by the different systems of an organism or the presence of toxic substances or its metabolites in the body. For example, instead of continuously measuring trace metal concentrations in sediment, water and biota, the concentration of the metal binding protein metallothionein can be measured in tissues of organisms, or instead of measuring tissue concentration of organophosphorous pesticides, it can be assessed through cholinesterase inhibition in blood samples (Handy *et. al.*, 2003).

Biomarkers of exposure can be divided into two groups

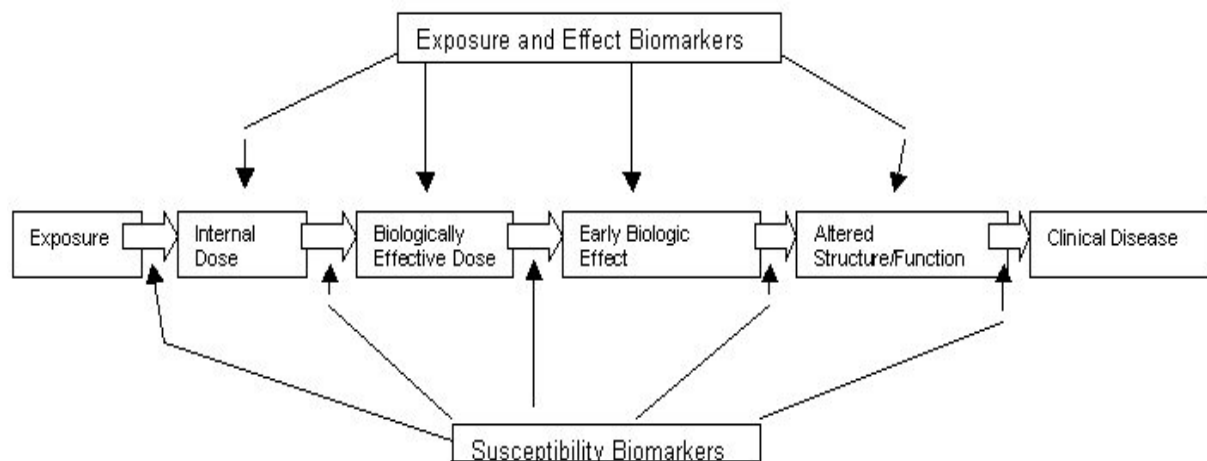
1. Biomarkers of internal dose
2. Biomarkers of effective dose

Biomarkers of internal dose give a measure of the occurrence of a toxin in an organism. This is measured as the amount of toxin or its metabolite in the system or in body fluids. Metabolites derived from glutathione (GSH) conjugation are one of the most important groups belonging to this category. GSH detoxifies reactive chemicals to which biological systems are exposed and are excreted as sulphur or glutamic acid-containing metabolites (Timbrell, 1998). Biomarkers of effective dose measure the interactions of toxicants and some specific molecular or cellular targets in the body such as protein receptors (Schmidt, 2006).

Biomarkers of toxic response or toxic effect are measured as health impairment or as early disease precursors or as a disease. They can be described as different cellular responses following exposure to toxic chemicals.

Biomarkers of susceptibility measure the variability expressed by different individuals when exposed to the same chemical or same environmental stress due to their differences in genetic makeup. Biological or genetic differences make some individuals more susceptible to environmentally induced diseases than others. A number of enzymes

or other molecules exhibit polymorphisms and this gives a measure to the magnitude and type of this response (Gulumian *et al.*, 2007).



(DeCaprio, 1997) (Figure 1)

Some advantages of biomarkers

1. they indicate biologically available contaminants rather than the inert form of contaminants
2. if a biomarker measures the induction of enzymes (i.e., it shows that a defence mechanism has been activated), and it involves energy expenditure that consequently affects an individual's reproduction and growth, this can translate into a population-level effect seen in the next generation (Walker, 1998)
3. a suite of biomarkers can indicate exposure to unknown contaminants from the environment
4. they can detect persistent contaminants in biological systems after they have been degraded in the environment
5. biomarker analyses are inexpensive in many cases and may be easier to perform than other chemical analyses (Handy *et al.*, 2003)

When an organism is exposed to increasing levels of a toxicant, it passes through three distinct physiological stages:

1. the stressed state and physiologically compensable state;
2. a phase in which a toxic effect is seen because the organism's ability to compensate is exceeded, but in which the effects are reversible; and
3. a stage where the damage is irreversible and death may occur.

The different physiological stages of xenobiotic chemicals effects in the body of an organism can be measured by looking at different biomarkers. The best biomarker is one that can detect and convey the presence of toxins at the first physiological phase. But it is desirable to have biomarkers that can detect the presence of toxin at least at the second phase.

Many types of biomarkers have been developed for different groups of animals including birds both in the field and in the lab, but most are destructive. Biomarkers that depend on collecting eggs from active broods or killing animals to obtain organs and tissues are known as destructive methods. Biomarkers can be assessed non-destructively too because chemical contaminants can affect both morphological and behavioural traits of an organism (McCarthy and Secord, 2000; Bortolotti *et al.*, 2003). So the presence and effects of contaminants can be effectively assessed non-destructively in birds by

evaluating their morphology and behaviour. Other non-destructive biomarkers can reflect the concentrations of contaminants evaluated in organs or tissues of animals found dead, or in feces, feathers, fur, skin and blood of live animals, and in abandoned eggs (Fossi, *et al.*, 1999; Marsili *et al.*, 1996), by assessing the quality of some of their ornamental traits, or by observing and quantifying some of their daily activities and behaviours. Nondestructive biomarkers are preferable in many ways.

1. They are necessary when rare, protected, or threatened species are to be studied and it is an important measure in wildlife conservation (Walker, 1998)
2. They permit simple and quick sampling, with minimal stress to individuals and population
3. They are useful when repeated sampling is required over long periods (Hatch *et al.*, 2002; Casini *et al.*, 2003).
4. Large number of individuals can be studied without population reduction (Walker, 1998)
5. They are ethical and humane

Materials that can be obtained non-invasively for biomarker analysis include excreta, abandoned eggs, shed feathers, breath (exhalation) etc. Blood, live feathers, skin and secretions of preen glands can also be used for biomarker estimation but here the procedure is invasive but nondestructive. This chapter reviews some of the materials that can be collected non-destructively in the laboratory and in the field for birds that live in organochlorine-polluted areas. Then there is a section that deals with semi-invasive procedures and tissues collected semi-invasively and their use in biomarker study. There is a section that deals with biomarkers and biological indicators which are measured in live birds such as morphological measurements and recording of reproductive success. Another section includes mostly biological indicators which are mainly behavioural changes noticed in polluted habitats. These sections are interspersed because some of the tissues can be collected semi-invasively and also nondestructively (ex. feathers) and some biological indicators and biomarkers are indistinguishable. The two most important

organochlorine groups included in this review are DDT and its metabolites, and PCB congeners.

Non invasive procedures

These are biomarkers that can be estimated in materials and tissues such excreta, shed feathers, abandoned eggs, morphological measurements, expiratory gases etc. Biomarkers can also be estimated by determining reproductive rates, growth of the individual or examination of external features like pigmentation of feathers, beak and feet and general appearance of external features and also their behaviours e.g., nest building patterns, nest attentiveness, nest abandonment etc). Additionally, ectoparasite load on individuals or in their nests can be measured. Of the biomarkers described below, some are already used in the field and in the laboratory and some are in early stages of study and use. Some of the described ones are biomarkers of exposure whereas others are indicators of effects. Most these need more research to refine them and to demonstrate their effectiveness and acceptability.

Feathers

Feathers can be used to directly estimate the presence and concentrations of organochlorine contaminants (Jaspers *et al.*, 2006; 2007; 2008). Feather pigmentation pattern can be used as a qualitative tool (Eeva *et al.*, 1998; Eeva *et al.*, 2008; Dauwe and Eens, 2008) and concentration of pigments can be used as a quantitative tool for estimating the presence of contaminants in birds (Dauwe and Eens, 2008). Feather colours can represent either the expression of pigments, or the refraction of light caused by the structure of the feather or by a combination of both (Gray, 1996; Shawkey, *et al.*, 2006). There are three different kinds of pigments found in birds - melanins, carotenoids and porphyrins. Melanins give rise to colours like darkest black to reddish browns to grey and tan. Melanocytes are formed in the neural crest and migrate to the dermis and supply melanosomes to the growing feathers (Rutz, *et al.* 2004). Melanin is formed by metabolism of the amino acid tyrosine (Fox, 1935). Birds must acquire carotinoids through their diet either by directly eating plants or indirectly by eating animals that have consumed plants (Gray, 1996). The colour of carotinoids ranges from bright yellow to

yellow and from light orange to bright orange. So quantity and quality of carotinoids in the diet, the proper digestion, absorption and metabolism of this pigment is important in maintaining the feather colour of that particular individual's feathers. Porphyrins are intermediate metabolites of heme synthesis produced by modifying amino acids. Porphyrins are responsible for a range of colors, including pink, browns, reds, and greens (Gray, 1996). Consequently, any contaminant-induced interference in the formation of melanocytes during development, carotinoid acquisition or metabolism, or heme metabolism can affect the colour pattern of feathers.

McCarthy and Secord (2000) suggested that examination of variations in ornamental traits was an efficient method to evaluate effects of some chemical contaminants in the environment. They studied patterns of plumage colour in subadult female tree swallows in their first breeding season and found that subadults in colonies in contaminated areas had significantly more adult-type blue-green color than did subadults from other sites. Bortolotti *et al.* (2003) exposed captive American Kestrels to dietary PCBs and observed them during breeding and winter seasons. At both times they observed sex specific differences in both colour and carotenoids between controls and PCB-exposed birds.

Dauwe *et al.* (2005) and Jaspers *et al.* (2007) estimated the presence of PCBs and organochlorine pesticides in bird feathers. Dauwe *et al.* (2005) could detect most of the PCB congeners and DDT and their metabolites that occurred in fat samples of 27 adult great tits in their feathers, also. Furthermore, the concentrations of organochlorines detected in the feathers were significantly positively correlated to what they could detect in the fat samples collected during breeding season. Jaspers *et al.* (2007) could detect PCBs and DDTs in single tail feathers of common moorhen (*Gallinula chloropus*). After analyzing eight different terrestrial and aquatic species, they recommended feathers as a non destructive biomonitoring tool, although they couldn't predict the exact body burden from the observed contaminant concentrations in the feather samples. Behrooz *et al.*, (2009) analysed museum preserved tail feathers of 37 birds belonging to 18 species from Persian Gulf region. They could identify many organochlorine pesticides such as DDT and its metabolites, hexachlorobenzene (HCB), α , β and γ -hexachlorocyclohexane (HCH) isomers, as well as PCB congeners.

Although the use of feathers in biomarker studies for the accumulation of organochlorines has received limited attention, feathers are widely studied to document metal contamination (reviewed by Burger, 1994; Dauwe *et al.*, 2000; Janssens *et al.*, 2001).

Despite the use of feathers in a broad variety of studies including ecotoxicology and chemoeology (Bortolotti, 2010) there are some constraints on their use as biomarkers of impairment, especially such qualitative attributes as colour pattern, brightness and hue. Because the feathers are exposed to environment, aberrations can be caused by physical and chemical conditions prevalent around the bird, and changes can be caused by external forces rather than from inherent causes. Fading of colours can be due to PCB or other contaminant chemicals in the feather or it can be due to acidic bleaching agent from the atmosphere adhering to the surface, or other exogenous materials especially when analysis are done on shed feathers.

Bortolotti (2010) recently reviewed many pitfalls in the chemical analysis of feathers. He proposed that instead of using a mass-dependent measurement of contaminants or other substances (elements and chemicals) in the feather it is better to use a time dependent measure because the growth rate of feathers and time available for deposition of materials into the feather from circulating blood depends on the growth stages and the amount of time the feather has grown. He also suggests considering the variations in the chemistry of feathers from different regions of the body of a bird and also variations within a feather in all types of studies.

Doucet and Hill (2007) examined the differences between the feathers of long-tailed manakins from the wild and museum preserved for different aspects of plumage. They found that plumage reflectance and coloration were within expected range whereas the colour of the skin of both groups of specimens showed significant differences. They attributed many reasons for the differences that are noteworthy in biomarker studies, especially when analyses are conducted with preserved feathers. They suggested that possible sources of altered colour in museum specimens are due to the use of preservatives, wearing off coatings by uropygial oil, and powder down secretions, excessive washing of soiled feathers with detergents, covering of feathers with dust and other chemicals from dirty habitats or from storage locations or mechanical disruption of

feathers by physical handling or by feather degrading bacteria or other pests etc. Thus it is important to know the physicochemical conditions of the habitat also before comparing feather types of different bird types, or birds from different habitats in biomarker studies.

Breath

The induction of cytochrome P4501A (CYP1A) can be used as a biochemical endpoint to measure the presence of polyhalogenated aromatic compounds in birds. The induction of CYP1A is measured in liver microsomes. It is measured as the rate of catalysis of CYP1A substrates such as ethoxyresorufin or activities of ethoxyresorufin - *O*-dealkylase (EROD), benzyloxyresorufin-*O*-dealkylase (BROD) or pentoxy benzyloxyresorufin-*O*-dealkylase (PROD) (Elliott *et al.*, 1996; Smits *et al.*, 2000; Custer *et al.*, 2001; Papp *et al.*, 2005) also in the liver. However, birds must be sacrificed to obtain liver samples. But CYP1A activity can also be measured by caffeine breath test (CBT) through use of radiolabelled substrate (^{14}C -caffeine) (Feyk and Giesy, 1996; Feyk *et al.*, 2000). Caffeine is an alkaloid and a xanthine derivative having methylation in N-1, N-3 and N-7 positions. During the oxidation process of caffeine the demethylation happens at all three positions. The N-3 demethylation of caffeine is a cytochrome pP450 (CYP 1A2) dependent reaction where the methane group will be converted to formaldehyde, formate, bicarbonate and exhaled as carbon dioxide. The demethylation of caffeine is a measure of CYP1A2 activity (Lambert *et al.*, 1993; Chung *et al.*, 1998). So if N-3 position is radiolabelled it will give a measure of the activity which happens in the liver. Feyk *et al.* (2000) used radiolabelled substrate (^{14}C -caffeine) to measure in vivo CYP1A activity twice during the development of common tern chicks fed for 21 days with fish spiked with different amounts of 3,3',4,4',5-pentachlorobiphenyl (PCB 126) and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153) such that the diet contained an average of 23, 99 or 561 pg of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents per gram fish. The CBT successfully detected PCB treatment differences by the presence of caffeine *N*-demethylation especially during week one. But there was greater induction of EROD activity than demethylation of caffeine. Numata *et al.* (2007) studied caffeine metabolism as a potent measure of CYP1A induction in two bird species, paradise shelducks and

southern black backed gulls. They concluded that caffeine metabolism was a potentially useful non-destructive biomarker for CYP1A induction in wild birds.

Caffeine metabolism is used as a biomarker in many vertebrates including humans as a measure of pollution and drug metabolism indicators. But this method has not attained much popularity with avian biologists because it needs more research into its usefulness in birds. Kennedy *et al.*, (2003) studied herring gulls for liver concentrations of PCBs and chicken embryo hepatocyte (CEH) bioassay-derived 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents (TCDD-EQs) and level of hepatic EROD and MROD activities and cytochrome P4501A (CYP1A) protein concentration. They found that the linear relationships between the concentration of chemicals and the liver chemical activity producing caffeine were not highly enough correlated to have much predictive power. So the authors cautioned against the use of EROD as good predictor of PCB pollution. Other researchers attempted to use caffeine as a probe substrate to examine the relationship between TCDD-dioxin exposure and CYP1A2 induction (e.g., Staskel *et al.* 2005). They found a very weak relationship between dioxin and CYP1A2 induction and concluded that dioxin may inhibit the catalytic activity of CYP1A2 (Staskal *et al.*, 2005). These results might have dissuaded researchers from using this very promising method as a nondestructive biomarker assay in the field and laboratory. To better establish the validity of this method more research is needed into its usefulness by assessing the underlying metabolic reactions.

Eggshell and membranes

One of the most serious effects of DDTs on avian wildlife is thinning of their eggshells, which is one of the most serious impediments to reproductive success (Lundholm, 1997; Gill *et al.*, 2003). Almost 40 years after banning the chemical from North America the effect of those chemicals are still evident in many parts of this continent. Shell thickness is an important transgenerational biomarker that can be evaluated to assess avian exposure to endocrine disrupting chemicals (EDCs) (Fossi *et al.*, 1999). This biomarker is known as “Index of Ratcliff”, which relates the DDT levels in maternal tissue to eggshell thickness (Peakall, 1992; Fossi *et al.*, 1999).

Chorioallantoic membranes (CAM) have been successfully used by many researchers to estimate maternal contaminant exposure and predict chemical concentrations of offspring in multiple species of birds and reptiles (Cobb *et al.*, 1995; 2003; Pastor *et al.*, 1996; Barger *et al.*, 2003; Pepper *et al.*, 2004). This highly vascularised extraembryonic membrane is used by the developing embryo for respiration, nutrient transport, and waste storage. The membrane is discarded with the shell at the time of hatching (Pepper *et al.*, 2004). This can be collected and can be used for contaminant analysis. Cobb *et al.* (1995) detected DDTs and PCBs in the CAMs from great blue heron eggs. Pastor *et al.* (1996) studied pipping eggs of Audouin's gulls for the distribution of organochlorine pollutants among yolks, embryos, and CAMs and they tested the usefulness of CAM as an alternative material for predicting whole egg contaminant burdens. Their results showed that CAMs could provide good predictions only for hexachlorocyclohexanes, PCB52 and DDD, as those pollutants were almost completely excreted to the CAM. Barger *et al.* (2003) studied the effect of PCBs and endosulfan in chicken eggs, membranes, and adults and suggested that CAM is useful in estimating the biological response caused due to exposure to PCB

There are many limitations to this method. If one is unable to collect nonviable eggs, it is important to collect egg shells with CAM immediately after hatching. This requires constant monitoring of the nestlings, especially towards hatching time. If the shells fall on the ground or become contaminated by adhering nesting materials it is difficult to clean them. CAM is such a thin membrane that collection of an adequate amount material, especially from small eggs is a difficult task. The membrane should be collected soon after hatching. Otherwise,, the membranes get desiccated and lose their usefulness as a material for toxicological studies. Again, the use of CAM as a nondestructive tissue for analysis of different contaminants should be tested further. As suggested earlier, studies by Pastor *et al.* (1996) showed that they were good predictors only for those contaminants that are fully excreted to CAM such as hexachlorocyclohexanes, PCB52, and DDD.

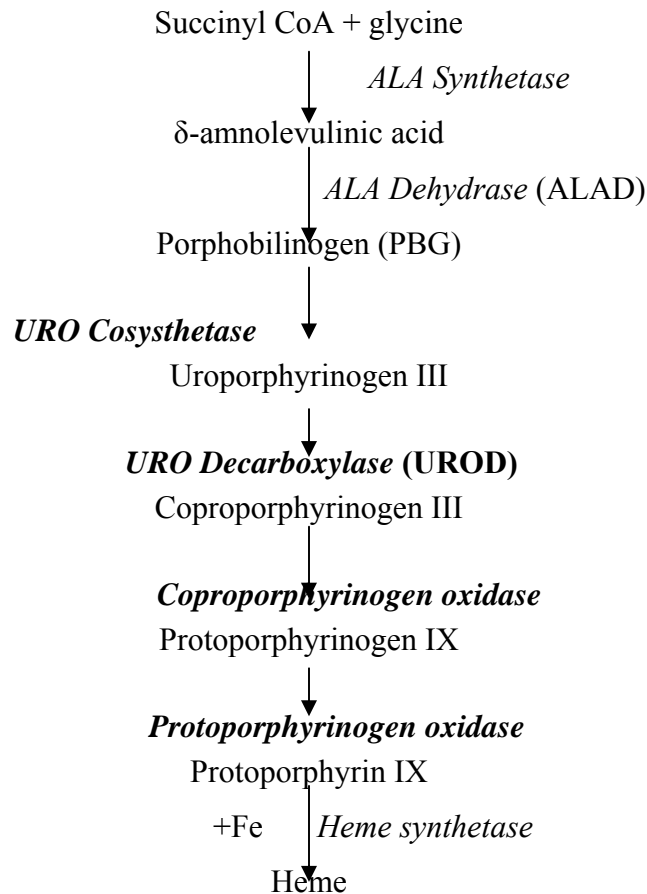
Feces

The excreta of birds is the cloacal mixture of feces and urine (Goymann, 2002). It consists of water, food residue, bacteria, nitrogenous waste products and secretions of the intestines and liver. Since it contains the liver secretions, it will accumulate all the associated metabolic waste products, pollutants and pollutant detoxification products. Therefore, feces are an ideal material to use for biomarker assays.

Many hormones and their metabolic byproducts can also be found in the excretory wastes. One such hormone is the stress hormone, corticosterone (CORT). Physical and chemical changes in the environment or any kind of stress to a bird can induce the production of corticosterone by affecting the hypothalamic-pituitary-adrenal (HPA) axis. Love *et al.* (2003) found that PCBs acquired through diet can induce fluctuations in corticosterone. Franceschini *et al.* (2008) studied adult female and nestling tree swallows exposed to different levels of contamination with polychlorinated biphenyls (PCBs) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the environment. They found that fluctuations in corticosterone levels reflected environmental contaminant concentrations. Goymann *et al.* (2002) concluded that birds' excreta can be used to analyze cortisone hormones instead of using blood plasma. So corticosterone measurements can be done noninvasively in excreta and semi-invasively in blood.

Another metabolite that can be measured in both blood and excreta is porphyrin, whose profile can be estimated as a biomarker of exposure and effect to organochlorines. Porphyrins are tetrapyrrolic pigments with a characteristic absorption spectrum that includes an intense band of absorbance at about 400 nm and a diagnostic spectrum that makes their detection and quantification both sensitive and specific (Smith, 1975; Casini *et al.*, 2003). Porphyrins are intermediate metabolites of heme synthesis. The heme part of hemoglobin consists of iron atoms that are attached to porphyrins. Porphyrins consist of five- numbered ring structures called pyrroles with interconnections (methane bridges) and side chains attached to pyrroles. The particular porphyrin found in hemoglobin is protoporphyrin IX, which can be distinguished from other porphyrins based on side chains - four methyls (-CH₃), two vinyls (-C=CH₂) and two propionic acids (-CH₂ - CH₂ - COOH). In the synthesis of heme, a glycine is activated by association with pyridoxal phosphate and combines with succinate, which has been in turn activated by association with coenzyme A. The activated glycine and succinate combine under the influence of an

enzyme called δ -amnolevulinic acid synthetase, to form δ -amnolevulinic acid (ALA). Two ALA molecules are combined to form the monopyrrole porphobilinogen (PBG) and four PBG molecules are assembled together to form the large, ring-shaped uroporphyrinogen. Following some modification of this ring molecule involving mainly the clipping of its projecting side chains, we arrive at the structure known as protoporphyrinogen IX and then protoporphyrin IX.



(Maclean, 1978; Peakall, 1992)

Some classes of environmental contaminants including PCBs can interfere with the synthesis of heme by directly interfering with enzymes (UROD or ALAD) of biosynthesis or by increasing the rate of oxidation of intermediary porphyrinogens. Other contaminants interfere with the enzymes uroporphyrinogen decarboxylase and coproporphyrinogen oxidase, resulting in the accumulation of Highly Carboxylated Porphyrins (HCPs). So porphyrins or their oxidative byproducts such as coproporphyrins and uroporphyrins or HCPs can be accumulated in blood, erythropoietic tissues (the liver

and kidneys) and are excreted via urine (Casini, *et al.*, 2001). Fox *et al.*, (1988) measured the concentrations of HCPs in liver tissues of adult herring gulls collected from the Great Lakes region during the early 1980s and found that they were positively correlated with the amount of halogenated aromatic hydrocarbons including PCBs in the environment. Japanese quails (*Coturnix coturnix japonica*) treated with Phenochlor DP6 and Aroclor 1260 showed porphyrin accumulation in the liver and other organs (Casini *et al.*, 2003). In tree swallow studies, Bishop *et al.* (1999) found that concentrations of uroporphyrin in the liver were correlated with amounts of PCB congener 118 in nestlings. Pulp and paper mill effluents have also been found to elevate HCPs in tree swallows (Wayland *et al.*, 1998). Kuzyk *et al.*, (2003) detected uroporphyrin, coproporphyrin and protoporphyrin in the livers of black guillemots exposed to PCBs in marine sediments, but they didn't find any variation with respect to Σ PCB exposure. Porphyrins can be detected in minute amounts, so they can be used as a very sensitive biomarker and possibly as an early warning signal of exposure in the laboratory and field (Fox *et al.*, 1988, Fossi, *et al.*, 1996). Porphyrins can be detected by a rapid fluorimetric assay or by High Performance Liquid Chromatography (HPLC).

Preen gland secretion (preen oil)

Preen glands or uropygial glands are located at the base of tail feathers in birds and secrete preen oil that is used for grooming and cleaning of feathers. They are found in almost all groups of birds. In passerines, the preen oil's most important components are mixtures of homologous monoesters made up of long chain acids and alcohols (Haribal *et al.*, 2005). Yamashita *et al.* (2007) estimated PCB concentrations and profiles in the preen glands and corresponding abdominal adipose tissue collected from 30 seabirds (2 orders, 3 families, 10 genera, 13 species) and found a weak but significant correlation between the PCB concentrations in the oil and abdominal adipose tissue. The authors collected preen gland oil (~50 mg) by wiping the gland with Kimwipe® paper tissues.

Reproductive parameters

Even low doses of PCBs and other persistent organochlorine contaminants, can disrupt the endocrine system and impair vertebrate reproduction and development (Colborn and Smolen, 1996). These compounds are neurotoxic, so they can induce changes in behaviour (Walker, 2003), physiological changes, and functional differences (Tanabe, 2002). Many chemicals appear to mimic vertebrate steroids, and interact with steroid receptors (Ottinger *et al.*, 2001). These chemicals can lead to reduced egg production, change in egg size, eggshell thinning, decreased fertility (Lundholm 1997; Verboven, 2009) and are particularly harmful during the embryonic, foetal and early postnatal periods (Fossi, *et al.*, 1999). Sublethal doses in adult birds can reduce parental attentiveness and cause abnormal reproductive behaviour (Barron *et al.*, 1995), ultimately resulting in reduced reproductive success. So the variables of reproductive success are important ultimate measures of the effects of chlorinated hydrocarbon contaminants in birds (Smits, *et al.*, 2000; 2005). This can be measured from nest building behaviour to all different stages of reproduction. Reproductive parameters that can be measured are effects on fertility (Barron *et al.*, 1995), date of clutch initiation (McCarthy and Secord 1999b; Fernie *et al.*, 2001), clutch size, sequence length (the length of time to complete the egg laying), brood size (Gill *et al.*, 2003), incubation period, hatching success, sex ratio, nest abandonment, burial of eggs in the nest lining (McCarthy and Secord, 1999b), feeding rates and amount, nestling period, nestling development and fledging success.

Nest building behaviour (biological indicator) of birds is initiated and enhanced by hormones. Nests are ephemeral but are made with greatest creativity and care to ensure that their eggs and young are getting ultimate comfort, adequate warm environment and protection during development. Tree swallows line their nests with feathers. The number of feathers in the nest lining will determine the level of insulation of the nest cups and thus ensure better growth of nestlings and protection from ectoparasites (Lombardo *et al.*, 1995). So another behavioural aspect that can be assessed is the number of feathers in the nest lining in different nest boxes in different sites. McCarthy and Secord (1999a) found that the number of nest-lining feathers at first egg, the number of nest-lining feathers at the last egg and the number of nest-lining feathers at hatching were significantly higher at uncontaminated sites than in PCB-contaminated

sites. Even though qualitatively, nest construction was similar at all sites, with a tightly woven grass mat filling the bottom of the nest liner and a nest cup lined with feathers, the nests at control sites had greater mass and more feathers than two contaminated sites, and also varied with PCB contamination of sites. Their observations confirmed that the behaviors of parents were affected by PCB contamination.

Many studies have reported the toxicity of dietary PCBs expressed as reproductive and developmental impairment including effects on fertility, egg production, hatching success, and chick growth. Verboven *et al.* (2009) found that there is variation in the allocation of resources to eggs by mothers exposed to pollutants. They found that females of *Larus hyperboreus* who had high levels of different organochlorines in their plasma laid smaller eggs, and egg size correlated negatively with the amount of pollutants. Rutkowska and Cihon (2002) reported that brood sex ratio of zebra finches was male-biased when females received low quality food and also when food quantity was poorer. The quality of food will be lowered when females are exposed to pollutants so that it could lead to skewed sex ratio.

Male Japanese quails treated with PCB hydroxylated congener 30 had a longer latency to mount the control females and were significantly less successful in copulation than reference males (Ottinger *et al.*, 2001). Studies on tree swallows by McCarthy and Secord (1999b) in several PCB contaminated sites along Hudson River found that reproductive success at PCB contaminated sites was significantly impaired due to high levels of nest abandonment during incubation and reduced hatchability of eggs. Halbrook and Arenal (2003) monitored the accumulation PCBs in European starlings (*Sturnus vulgaris*) at nest boxes constructed at two PCB exposed and two reference sites during the breeding season and observed their productivity (number of chicks produced per nest) and adult nest attentiveness behaviour (provisioning behaviour). Reduced adult nest attentiveness behaviour and decreased chick survival were observed at PCB sites. Nest success was substantially reduced in a tree swallow population breeding in chlorinated hydrocarbon-contaminated habitats due to nest abandonment by parents (Harris and Elliot, 2000). Reproduction of captive American kestrels was suppressed when adult birds were exposed to PCBs (Fernie *et al.*, 2001). Hernandez *et al.* (2008) studied temporal and regional trends of OC pesticide and PCB levels in eggs of the Spanish

Imperial Eagle (*Aquila adalberti*) collected in Spain between 1972 and 2003 in three populations central, western, and Don~ana (Don~ana being the most polluted of three) and found that significantly lower values for egg volume and breadth as well as Ratcliffe Index after DDT use than during the pre-DDT period; and eggs were significantly smaller when DDE levels increased. Clutch size in Don~ana varied according to DDE concentrations with the highest DDE concentrations found in clutches consisting of one egg. The fertility rate was lowered in clutches with DDE levels greater than 4.0 µg/g. This was a consequence of hatching failure and fewer fledglings.

Many studies have found that reproductive rates of tree swallows are reduced by organochlorine contaminants (Custer *et al.*, 1998; 2003; 2005; McCarthy and Secord 1999a, Bishop *et al.*, 1999). Studies by Fernie *et al.* (2001) on American kestrels suggest that there was reproductive loss through the mortality of nestlings whose parents had been exposed *in ovo* to PCBs. The mechanism was suggested to be altered parental behaviour or reduced nestling viability. Neurotoxic compounds can reduce the ability of prey species to avoid predation. Such chemicals also lead to aberrant behaviour that can attract the attention of predators (Walker, 2003). Thus, overall survival and success of the affected population will be impaired by these kinds of contaminants. Consequently, it is necessary to assess the status of the population and to develop definite nondestructive biomarkers from the list of parameters available that can be used to detect the exposure of a population or populations to these contaminants.

Morphology and growth

Exposure to PCB and other organochlorines can result in decreased growth because both hypothalamo-pituitary and thyrotropin-thyroid axes required for normal growth can be impacted by organochlorine pollutants (Gould *et al.*, 1997). Many studies have found that the thyroid axis has been affected by PCBs. An alteration in growth can affect all morphological features. Effects of PCB 126 on chickens, American kestrels and common terns were studied by Hoffman *et al.* (1998) from eggs to hatching after air cell injection on day 4, found beak defects in all three species. Defects were observed at a concentration of 0.3 ppb in kestrels. Defects were noticed in other two species at a higher concentration than in kestrels. Bustnes *et al.* (2002) noticed a correlation between

organochlorine pollutants and wing feather asymmetry in Arctic breeding Glaucous Gulls (*Larus hyperboreus*, Gunnerus). They found asymmetry in the lengths of the third primary in the left and right wings. The authors suggested that fluctuating asymmetry could serve as a potential measurement in predicting the early effects of organochlorine pollution on bird populations.

Ectoparasites

Another condition that can be assessed in relation to biomarker/biological indicator studies is to enumerate the numbers of associated common ectoparasites such as fleas (Siphonaptera) and blow fly larvae (Calliphoridae: Diptera) (Thomas and Shutler, 2001). These insects are commonly found in bird nests, on nestlings and on adult birds. Gentes *et al.*, (2007) studied tree swallow nestlings for the infestation of blow flies *Protocalliphora* spp. (Diptera: Calliphoridae) in 38 nest boxes distributed in oil sands reclaimed wetlands and reference areas near Fort McMurray, Alberta, Canada. They found that all the 38 nests were infested, but the magnitude of infection was about two times higher in nestlings whose boxes were situated within oil sands reclaimed wetlands compared to reference areas. The nest materials on oil sands reclaimed region carried very high parasitic load (with a range of 60% to 72%) compared to reference nests. They also found that the infested nestlings were growing less efficiently. This is a very easy assessment that can be done with minimal disturbance to birds. Ectoparasites can be checked in the nest cavities by inserting hand wearing white gloves.

Bird song

Bird populations are sensitive to PCBs in the environment as they can cause neurological damage, and this can affect their overall behaviour (Walker, 2003). One such behaviour that can be affected is bird song. DeLeon and Dhondt (2008) found that song quality of black-capped chickadees (*Poecile atricapillus*) was impaired by the presence of PCB. They looked at differences in the interval ratio (a measure of song quality) among locations (those with PCBs present vs. reference sites), and the interval ratio variation between and within individuals. They found that the average interval ratio was lower in the low PCB site ($P < 0.001$) than in the higher PCB site. On this basis, they

suggested that song learning but not song production appears to be affected by PCBs. Their research provided a possible non-invasive bioindicator of effects for sublethal PCB levels in passerines.

Semi-invasive biomarkers

Skin

Residues of persistent chemicals and CYP450 can be measured in skin samples taken from animals and birds (Walker, 1998). A less invasive technique is the phytohaemagglutinin skin-testing technique of avian immunocompetence (Smits *et al.*, 1999; 2002). This test evaluates the proliferation, differentiation and response potential of circulating T-lymphocytes to an injected mitogen (Smits *et al.*, 1999; Grasman, 2002). When PHA is injected into skin, a swelling will develop in the injected area within 12- 24 h. due to proliferation and differentiation of T-cells, cytokine production and accumulation of other white cell corpuscles and fluid. In the past, this was compared with the effect of a placebo treatment, i.e., an injection of phosphate buffered saline (PBS) into an alternate site. However, recently researchers are omitting this step to avoid extra stress to the organism undergoing the tests (Smits *et al.*, 1999; Grasman, 2002). In birds the injections can be made into wing web, wattle, dewlap or interdigital skin (Grasman, 2002). Smits *et al.* (2002) found that PCB-exposed American kestrels had a greater response to PHA than controls, when analysis was done with sex as a significant factor and plasma triiodothyronine (T₃) as a significant co-variate.

Biomarkers in blood

Blood can be obtained easily from birds (invasive but non-destructive) and many biomarker assays can be performed on blood. Clinical haematology and biochemistry are valuable tools in assessing the health of birds (Sergent *et al.*, 2004). Hematological variables that can be used as indicators of immune structure and function and the health status of birds include total red blood cell count, total leucocytes (WBC), % heterophils, % lymphocytes, % monocytes, % eosinophils and their ratio to one another (Grasman *et al.*, 1996; 2000a).

DNA alterations including strand breakage, adducts and sister chromatid exchanges (SCE) can be detected in blood cell nuclei (Gil and Pla, 2001). Blood can also

serve as a biomarker for poly aromatic hydrocarbon (PAH) induction. Protein responses including mixed function oxidases (MFO), protein inductions and stress proteins, haemoglobin adducts, various enzyme inductions and presence of aminolevulinic acid dehydratase (ALAD) are another suite of biomarkers that can be detected in blood as a consequence of PAHs exposure in birds. Presence of metabolic products like porphyrin and other by-products of heme synthesis and immunological changes can also be detected in blood following exposure to PAHs (Fossi, *et al.*, 1999).

Immunological changes (immunomodulation):

Different studies have proved that immune system is very sensitive to environmental contaminants (Grasman *et al.*, 2000a; b; Grasman and Fox, 2001; Smits and Bortolotti, 2001; Grasman, 2002; Smits *et al.*, 2002; Finkelstein *et al.*, 2003; Holloway *et al.*, 2003). Exposure to PCBs and other organochlorines from the environment makes all animals including birds more susceptible to many disease-causing epizootics. This is because the toxic chemicals modulate the immunology of organisms and this will lead to reduced resource availability for many other physiological functions including resistance to disease and infections (Wong *et al.*, 1992; Grasman, 2002).

An organism's immunological status can be assessed by the mass, volume and structure of immunological organs like thymus, spleen and bursa of fabricius and blood cells (Grasman, 2002). Tissues other than blood cells require sacrificing of the animals but blood cells and blood chemistry can give faultless information on the status of immune state of an animal. A very simple and reliable method to assess immune state of an organism in the blood is by cell counts (Grasman, 2000a). Different white blood cells (WBC) are important in cell-mediated, antibody-mediated and non-specific immunity. Total WBC and differential counts are reliable indicators of immune status and general health of organisms. A variation in the number of a type of WBC shows that there is some variation in the health of an organism and in most cases it indicates that the organism is fighting an infection. Grasman *et al.*, (1996, 2000b) observed elevated ratios of heterophils to lymphocytes in Caspian tern chicks exposed to dioxin-like chemicals including PCBs. Heterophils and lymphocytes are the most numerically abundant WBCs in avian circulatory system. Grasman and Fox (2001) in a study with young Caspian terns

found that plasma PCB and DDE concentrations and percentages of monocytes were negatively correlated and percentages of basophils and the concentrations of chemicals in plasma were positively correlated. Bustnes *et al.*, (2004) counted heterophils and lymphocytes and their correlation with blood concentrations of organochlorines (p,p'-DDE and 7 different PCB congeners (PCB 101, 99, 118, 153, 138, 180 and 170)) in Glaucous gulls (*Larus hyperboreus*) in 1997 and 2001. They found significant or near significant ($0.1 > p < 0.001$) positive relationships between the OCs and levels of heterophils in the blood for both sexes in 1997 and for male gulls in 2001. The levels of lymphocytes and OC concentrations were positively correlated in both sexes in 1997.

Serum chemistry values that can be assessed to evaluate the immunological status include ionic content (calcium, phosphorus, sodium, potassium, magnesium, iron, chloride etc.), glucose content, uric acid, cholesterol, enzymes like amylase, alanine amino transferase (ALT), aspartic amino transferase (AST), creatine kinase (CK), alkaline phosphatase, sorbitol dehydrogenase etc., total bilirubin, total CO₂, and total serum proteins (albumin and globulin and their ratios) (. Relative and total amounts of plasma α , β , and γ globulins are affected by different types of infections, inflammations and by physiological conditions of an organism. Grasman *et al.*, (2000a) found that in pre-fledging herring gulls from Great Lakes region concentrations of β_2 globulin increased as a function of PCB and DDT levels in the tissues. In terns, PCB concentrations were negatively correlated with α globulins and positively associated with β_1 globulins.

There are *in vitro* and *in vivo* tests available to test birds' immunological functional levels. The most commonly used *in vivo* immune tests in birds are the phytohemagglutinin (PHA) for T-cell mediated immunity (Grasman and Fox, 2001; Grasman, 2002; Smits *et al.*, 2002) and sheep red blood cell (SRBC) hemagglutination assay (Grasman and Fox, 2001; Grasman, 2002) or dinitrophenol-keyhole limpet (DNP-KLH) test (Smits *et. al.*, 2001) for antibody-mediated immune responses. Grasman and Fox (2001) in their study (mentioned before) with young Caspian terns found that there was a strong negative correlation between PHA response and plasma PCB and a lesser degree of negative correlation with DDE in the plasma. But the total antibody titres following immunization with SRBC showed positive association with plasma PCB and DDE.

Phagocytic properties of white blood cells such as macrophages can be used as another potential immunological indicator (Fournier *et al.*, 2000). Lymphocyte proliferation and phagocytosis can be performed *ex vivo* in cryopreserved avian peripheral blood cells and can be performed in a single blood sample (Finkelstein *et al.*, 2003).

Blood collection is not favoured by many bird lovers as the technique is invasive and can cause distress to birds. Becker *et al.*, (2006) suggested a non-invasive method to collect blood from breeding birds. A larval instar of a blood-sucking bug *Dipetalogaster maximus* (Heteroptera) was introduced into common tern (*Sterna hirudo*) nest within a hollowed artificial egg. The hole was covered with gauze so that the bugs could collect blood from the brood patch of breeding adults. This can be done without trapping or handling the breeding birds. The authors claimed to have collected sizable amounts of blood by the above method.

The presence of plasma esterase activities in blood is another indicator and signal of potential problems of organophosphate (OP) and carbamate pesticides (Strum *et al.*, 2008; Peakell, 1992). These pesticides act directly on the nervous system by inhibiting acetylcholine esterase (AChE). AChE is the enzyme that helps in the Acetylcholine (ACh) mediated synaptic transmission. ACh is a neurotransmitter in both the peripheral nervous system (PNS) and central nervous system (CNS) in many organisms including humans and also one of many neurotransmitters in the autonomic nervous system (ANS) and the only neurotransmitter used in the somatic nervous system.

Problems with biomarker research

There are many options available to biomarker researchers but there are many unresolved problems, too. One major source of confusion in the field is the proliferation and user of vague definitions with no clear cut distinction being made in the use of the terms biomarker, biological indicator and ecological indicator. Different researchers apply different criteria for choosing their biomarker. Forbes *et al.*, (2006) excluded whole-organism response, survival and reproduction from their biomarker category, whereas Jamil (2001) included all the above along with behavioral changes in his biomarker definition. While some authors restrict their use of the term biomarker to

measures of toxic effect produced by a xenobiotic substance at the individual level or below (Mayer *et al.*, 1992; Walker, 1998) others like Peakall (1994) extrapolated it to structure of communities and ecosystems. van Gestel and van Brummelen, (1996) explicitly defined the three terms, but still the terms are used vaguely by many researchers. This makes it hard to strictly separate different levels since they are all overlapping entities.

Forbes *et al.*, (2006) described many uses and misuses of biomarkers in ecotoxicology. They pointed out the need to distinguish whether measured biomarkers are indicators of exposure or of effects. Effects biomarkers should have the capacity to consistently link to the effects produced at higher levels of biological organization. It is also important to distinguish the effect produced by an organism due to contaminant interaction from effects produced by an organism due to interactions to other environmental conditions. Sometimes organisms show certain effects due to their interaction with the different physical conditions prevalent in the environment (temperature, geographical influences, habitat characteristics, etc.) or because of the physiological conditions (nutritional state, reproductive condition, genetic makeup etc.). A biomarker should be able to differentiate these effects.

An especially important aspect to be considered is the predictive power of biomarkers. The dose response curves of most existing biomarkers used in ecotoxicology are not linear. Some exhibit a diagnostic pattern (response) to a certain level of exposure, and then jump to an aberrant path. So it is necessary to have a suite of biomarkers that can all be linked together in an integrated manner to give the required power to predict ongoing effects (Forbes *et al.*, 2006).

Gil and Pla (2001) suggested that important attributes of biomarkers include ease of sample collection and analysis and specificity of effect. The response should be diagnostic of a specific exposure (like aminolevulinic acid dehydratase (ALAD) inhibition by lead). Experience suggests that for biomarker assays, if one step is easy and inexpensive, the other step will be complicated. For example, feather collection or blood collection are relatively straightforward processes but the steps that follow are complicated. For blood, the storage, transportation, preparations etc. (especially from field stations) are sometimes problematic. For feathers, collection, and transportation are

easy but the subsequent steps are more complicated. Specificity of effect is also an important property to be considered for biomarkers.

Summary, implications, and recommendations

Biomarkers provide a quantitative measure of bioavailable pollutants and their effect on biota that cannot be obtained by just measuring the amount of pollutants in the environment. The long term goal of biomarker research is to find a suitable end point that gives the presence of one pollutant or another at a very preliminary stage of exposure in the individuals of a population. It should be possible to extrapolate measured biomarkers to other populations so that any changes in the population and to their community can be visualized and management options can be put into place. Rapidly responding biochemical markers can work as indicators of any change, which if not monitored can lead to irreparable changes to the populations, community and ecosystem at large.

Biomarkers are important in the field of surveillance, biological conservation, environmental hazard assessment and regulations and ecological remediation (McCarthy and Shugart, 1990). Effective environment management requires knowledge of the movement and fate of contaminants in the different compartments the natural system (Shugart, 1990) including the physical, chemical and biotic compartments. The biomarkers are an integrated measure of the contaminants' effects in the biotic compartment. So biomarkers are very important in wildlife toxicology. Consequently, testing should involve minimal sacrificing of individuals of any population. In this review, I have identified many alternative biomarkers that can be used in the field. The task ahead is to refine those different methods and find a few which are best suitable ethically and economically and work realistically. An ideal biomarker should provide an early warning signal and best be able to predict the true situation of pollutants in the field. Another important aspect in ecotoxicology is to find suitable biomonitors or sentinel species - species in which we can test the biomarkers. The biomonitor species should be neither extremely sensitive to pollutants nor highly tolerant. Birds are excellent models for biomonitoring. They are easy to observe and are found in almost all habitats. They are sensitive to toxicants, occupy various trophic positions in the food web, and have well defined life histories and behavioural patterns. So an important task in the field of

ecotoxicology is to find a suite of biomarkers that are ethically acceptable, technically sound, environmentally correct and economically feasible.

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Chapter 7: General conclusions

Many studies recognize the importance of bioenergetics in contaminant accumulation in aquatic organisms, especially fishes (Beyers *et al.*, 1999; Madenjian, *et al.*, 2000; Trudel and Rasmussen, 2001; 2006). But fewer studies have dealt with bioenergetics and contaminant accumulation in terrestrial organisms. Although bioenergetics based contaminant accumulation in birds has been studied by some (e.g., Nichols *et al.*, 1995; 2004; Glaser and Connolly 2002; Dauwe *et al.*, 2006), this study is special in that it has compared the bioenergetic requirements and dietary composition of nestlings of three insectivorous migratory passerines developing in overlapping spatial niches in a common location (Point Pelee National Park). My work has demonstrated that the accumulation of persistent organochlorines is mainly determined by species-specific energetic requirements and the composition of their diet (i.e., the relative proportions of aquatic and terrestrial insects in the diet and the contaminant signatures and quantities of insects comprising each species' diet (Chapters 3 and 4). Nestlings' contaminant accumulation is also determined by the diet of the mother during and before egg formation and through the diet of nestlings during their post hatching period (Chapter 2). Another important determinant is the biotransformation and elimination capacity of the species (Chapters 4 and 5). This study focused on PCB congeners and DDT and its metabolites because evidently these two classes of organochlorine compounds still pose threats to organisms in Point Pelee National Park as they persist in the sediments and soils of the park and neighboring areas. These chemicals are extremely stable and can be easily mobilized to different compartments of the biosphere by the movement of different environmental media (Spears *et al.*, 2008).

Tree swallow nestlings are frequently used as bioindicators for the accumulation persistent aquatic sediment bound organic pollutants (Bishop *et al.*, 1995, 1999; Custer *et al.*, 1998; 2000; 2003; 2005; Custer and Read, 2006; Froese *et al.*, 1998; Secord *et al.*, 1999; Smits *et al.*, 2000; 2005; Brasso and Cristol, 2008; Jayaraman *et al.*, 2009), and house wrens are used as biomonitors for terrestrial soil bound pollutants (Neigh *et al.*, 2006; 2007). Purple martins are intermediate in their feeding of terrestrial and aquatic insects so are prone to accumulate pollutants from both of these habitat types. This

species has yet to be assigned a position in the literature on contaminant related bioindicator studies.

I undertook this study with the assumption that the nestlings of all three passerine species have no biotransformation capacity, and I developed a flow chart summarizing what I predicted to be the important determinants of bioaccumulation excluding biotransformation. Based on my findings, I have modified the flow chart. The revised flow chart has biotransformation as an additional determinant and also includes the prebreeding and breeding grounds of maternal birds as another determinant of nestling contaminant burdens, because my study of egg contaminant concentrations and signatures (Chapter 2) clearly indicates that some of the accumulation in the egg must have been acquired is from the breeding ground, too. All the determinants in contaminant bioaccumulation to avian nestlings are given in a flowchart (Figure 1).

Role of eggs in contaminant bioaccumulation

The eggs of all three species were more contaminated with Σ DDT and Σ PCB than the bird tissue contamination level observed in the park. The concentrations of Σ DDT were almost negligible in the diets of nestlings of all three species, so the contaminant burden of the egg had a more prominent role in the contribution of contaminants to the body tissues of birds at fledging. Mean \pm SE of 334.6 ± 93.9 ng/g DDT in the eggs ($n=44$) was higher than 135.2 ± 53.5 ($n=5$) observed in the tissues of house wren nestlings and for tree swallows, the egg value of 639.7 ± 116.6 ($n=4$) was higher than tissue level of 277.2 ± 186.5 ng/g DDT ($n=4$). Similar findings were reported by Dauve *et al.* (2006) in nestlings of great tits (*Parus major*). The growth dilutions observed in this study have also been described by various other researchers (Nichols *et al.*, 1995; Spears *et al.*, 2008). This reflects the rapid growth of nestlings and low contaminant concentrations in the diet (Neigh *et al.*, 2006). The Σ PCB accumulation in house wren nestlings were very low compared to the egg value but in other two species, fledgling tissue PCB concentrations were maintained at the same level originally observed in the eggs. The egg mass played a very important determinant role in Σ DDT and Σ PCB accumulation in house wren eggs, mainly because the egg constituted a relatively high

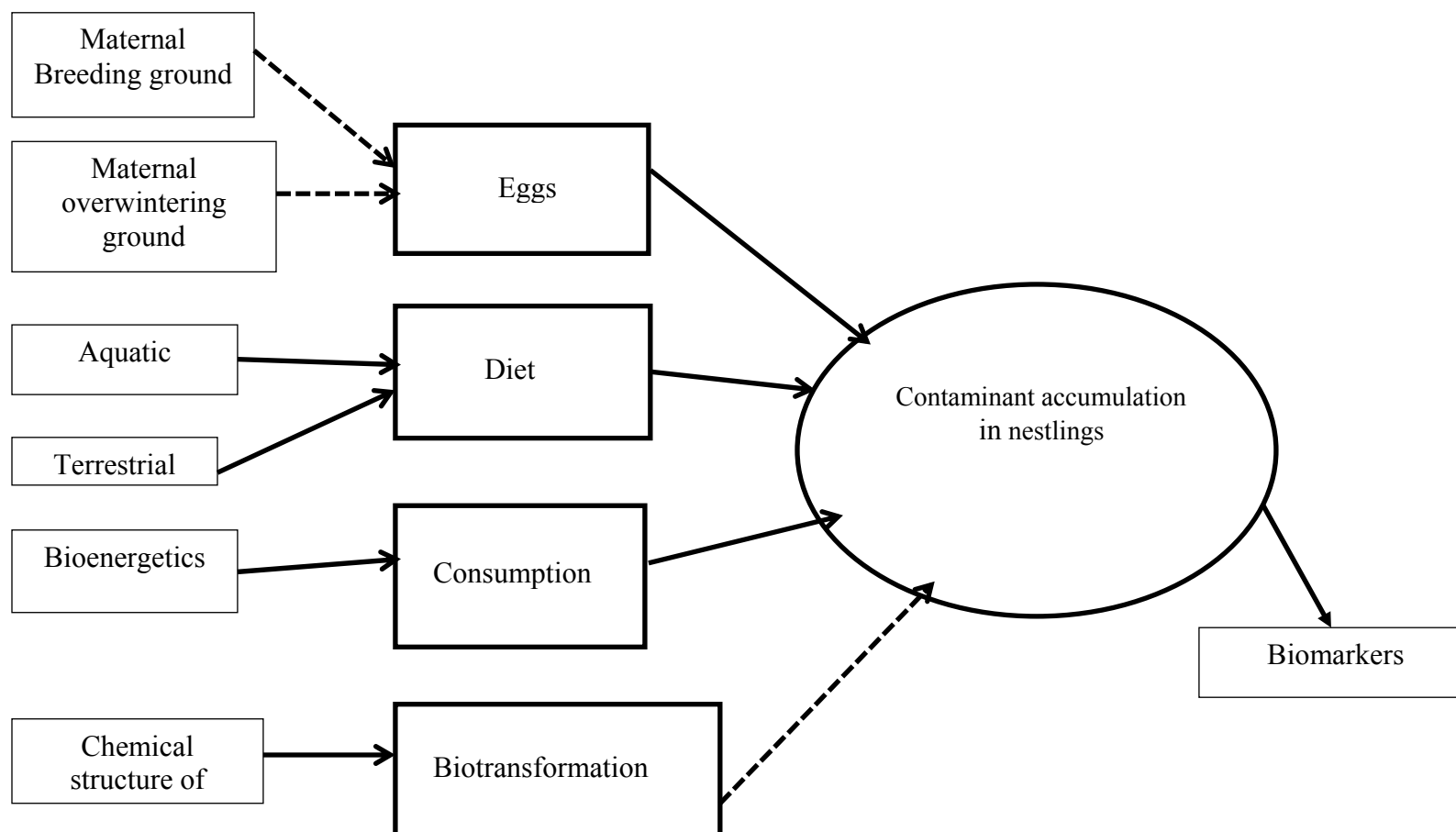


Figure 1. Square boxes and arrows highlight the important determinants in contaminant accumulation in migratory passerine nestlings. The dashed arrows came into prominence after this study

percentage of the nestling's mass on the day of contaminant determination or fledging weight. In house wrens, the egg mass was 14.95% of fledging mass, for tree swallows it was 7.08% and for purple martin it was 7.02%. When the contaminant contributions from the diet are minimized and when the egg weight is an important factor in the contribution of hatchling weight, egg burdens (derived from maternal contaminant load) play a larger role in contaminant contribution to the nestlings. The inter-specific differences in accumulation of different pollutants by the eggs are mainly due to the differences in their maternal diets (Gao *et al.*, 2009).

Egg contaminants can have been accumulated either from maternal foraging at their overwintering prebreeding grounds, from feeding at their breeding grounds (Point Pelee) or from food consumption at stopover places during migrations. Accumulation from the breeding ground could be estimated from the pattern of accumulation, i.e., accumulation and distribution pattern of readily cleared chemicals like some PCB congeners with open *meta-para* positions on their biphenyl ring and other organochlorines like dieldrin. There was considerable accumulation of different contaminants in tree swallow eggs from their breeding ground. This was inferred from the relatively high accumulation of PCB congeners that belong to the readily cleared category in their eggs.

Role of nestling diet in contaminant bioaccumulation

The results described in the third chapter (diet of three species) showed that there were significant differences in the ratio of aquatic to terrestrial insects in the diet of three species and a difference in the type of insect orders that composed both aquatic and terrestrial groups in their diet. In the house wren's diet Orthoptera (grasshoppers) and Aranaea (spiders) constituted the largest groups by biomass. These terrestrial arthropods are found among vegetation and so could be easily caught by gleaning wrens. The two major orders found in the diet of purple martins were Coleoptera (beetles) and Odonata (dragonflies and damselflies). Odonata was represented by the dragonfly family Libellulidae. Both insect orders contain strong-flying species, especially the dragonflies. The dragonflies in particular often fly high up in the air and will overlap with the flight

realms of purple martins. The two major orders represented in highest proportions in tree swallow diets were Diptera (Chironomidae) and Ephemeroptera (Ephemeridae: *Hexagenia*) - the adults of aquatic insects, which fly mainly in the areas in or close to the water bodies over which tree swallow parents are most active.

The differences in the 3 species' dietary compositions were reflected in the contaminant loads of Σ PCB and Σ DDT received by the nestlings from their diets. Clearly, the environmental contaminants accumulated by arthropods from their habitats are transferred to their consumers. The process of larval development and the habitats in which they develop influence the types of contaminants that they may accumulate and pass on to their predators. The birds' specific feeding strategies, (aerial hawking and gleaning) and the prey that they target are the most important factors that determine the nestlings' exposure dynamics (Sagerup *et al.*, 2002; Neigh *et al.*, 2006; Dauve *et al.*, 2009). The data from chapter 4 showed that Σ PCB was highest in swallow diet (258.6 ± 148.8 ng/g wet weight) followed by purple martins (101.9 ± 4 ng/g wet weight) and lowest in house wrens (24.5 ± 9.6 ng/g wet weight). Tree swallows had more than tenfold higher levels of Σ PCB than house wrens, reflecting the more than tenfold proportional difference in aquatic insects in the diet based on biomass (tree swallow, aquatic insects 82.4 ± 32 % and house wrens 7.3%, 2005 study). Σ DDT followed a slightly different trend than expected, with house wrens having the highest loading (30.6 ± 10.2 ng/g wet weight), followed by swallows (14.8 ± 3 ng/g wet weight) and purple martins with the lowest Σ DDT, ($10. \pm 1$ ng/g wet weight). Σ DDT of insects in the park (ants, midges and mayflies, 2 samples from each group of which one sample was from a contaminated and the other from a reference site, chapter 3; Smits *et al.*, 2005) were assessed in 2003. The aquatic insects were collected in the years 2002 and 2003, and ants were collected in 2003. Aquatic insects had a mean \pm SE of 27.2 ± 9.49 ng/g wet weight whereas the ants from the contaminated site had a value of 197.29 ng/g wet weight and reference site had a value of 7.24ng/g wet weight. The diets of nestlings for the present study were collected in 2005. Σ DDT content in the diet is low in all three species (compared to the previous study, too), showing that the banning of DDT use from the park has had an effect on Σ DDT bioaccumulation in insect populations of the park and thus the transfer to the higher level consumers.

As diet of nestlings is the most important determinant in contaminant bioaccumulation, the concentration of contaminants in the diet will be added up as the observed contaminant values in the tissues of nestlings. Determination of the contaminant concentration in the diets of three species gave us the power to predict the tissue loadings for those species in Chapter 4. Variations in the final accumulation were the result of the caloric content of the diet and the digestive, assimilative and absorptive efficiencies of different nestling species. The abundance and availability of adequate prey insects as food also is important in determining the overall accumulation of the contaminants and also the overall growth and performance of the nestlings.

Bioenergetics-based model

The goal of this chapter was to see the extent to which the nestlings' bioenergetics could be used to estimate the contaminant accumulation pattern along with the observed contaminant levels in the diet and the eggs of three different species. The growth rate and associated consumption rates for three species studied were different. The egg had a larger determinant role in contaminant accumulation especially in wren nestlings because of their higher egg weight relative to fledging weight compared to other two species.

We determined absolute growth of the nestlings and from there calculated the total consumption with the help of values of different parameters obtained from the literature. We used observed values of diet and egg concentrations to predict the accumulation of \sum DDT and \sum PCB and found that they broadly fell within the range observed in the tissues of fledglings of each species. The observed differences were mainly due to a lack of precision in some of the estimated parameters. For animals living in the wild, the consumption rate, the prey/food availability, the digestion and assimilation efficiencies *etc.* can vary over short periods of time due to changes in the abiotic factors that affect them. Values will vary among species, too, with the changes in their abiotic environment. Seasonal temperature is one such factor that can affect the total consumption rate (Drouillard *et al.*, 2009). Those changes will lead to inter-specific differences in contaminant bioaccumulation (deBruyn and Gobas, 2006). Because the nestlings in our study grew under fairly consistent environmental conditions and the nestling periods were of short duration, the models were applicable to them.

Another important aspect of contaminant bioaccumulation in nestlings is the metabolic biotransformation/elimination capacities. We assumed that nestlings had no biotransformation elimination capacity based on the tree swallow nestling studies of Nichols *et al.*, (1995; 2004) and Papp *et al.*, (2007). In order to estimate the biotransformation capacities, we classified the congeners into a readily-cleared group and a slowly cleared group based on the studies of Drouillard *et al.*, (2001; 2007) and Clark *et al.*, (1987). In order to estimate onsite accumulation we compared the observed values with amount of contaminants that could possibly be accumulated from food at the breeding locations. The readily cleared congeners showed different accumulation patterns across the three species, which specifically demonstrated their biotransformation capacity and onsite accumulation pattern. Wrens showed no evidence of biotransformation elimination as the predicted values were below the observed values, and exhibited very low accumulation of PCB congeners from Point Pelee National Park. Purple martin showed a different trend. They showed both biotransformation/elimination and onsite accumulation. In contrast, tree swallow nestlings exhibited a different pattern - onsite accumulation and no biotransformation capability. This confirmed the earlier studies on tree swallow nestlings by Nichols *et al.* (1995; 2004) and by Papp *et al.*, (2007). Slowly cleared congeners in all three species showed K_{ow} related absorption pattern and different levels of accumulation from breeding site.

DDE, which is also a slowly cleared organochlorine contaminant, showed the same tendency in accumulation as other slowly cleared congeners, especially in purple martins and tree swallows. House wrens showed slightly higher accumulation as their food in Point Pelee National Park was mainly terrestrial insects.

Of all the factors examined in this research, diet is the most important determinant in contaminant accumulation by birds. The diet will influence the total contaminant burden directly by feeding or indirectly through the eggs. The eggs in turn reflect the diet of the female parent. The dietary intake is determined by the energetic requirements of the individuals and this is reflected in the growth rate of the animal.

If a biomarker can be measured as a direct effect of a contaminant, the contaminant concentration of the tissues can be estimated from the biomarker's expression. The tissue concentration or the state variable is mainly influenced by the

bioenergetics of the species. So from the state variable of the model, the inputs can be estimated, including the environmental contaminant level. This is useful in ecological risk assessments and to estimate contaminant levels in other organisms living in those areas (Glaser and Connolly, 2002). Knowledge of the level of environmental contaminants alone will not give the critical information as to whether or not they are biomagnified or bioaccumulated. So a quantifiable biomarker will be a single test for contaminants at different levels, the environment, the food organisms in birds' diet, and the birds.

Feeding on contaminated mayflies and their effects

The feeding experiments were subject to many unforeseen obstacles in the field and consequently the overall performance of the experiment was below the expectations. There were significant differences between the Σ PCB content of different sources of mayflies used in the dietary manipulation study and also there were variations in tissue accumulations by different house wren nestling assigned to different feeding groups. There were no significant differences in Σ DDT among any of the mayfly treatments and feeding groups. The growth rates of the nestlings were affected by the amount of Σ PCB especially in the groups fed mayflies from Monroe, Michigan (the most contaminated of the natural mayflies collected). Their liver and kidney weights were affected, demonstrating that their detoxification mechanism had become activated. But this was not shown in their accumulation profile of readily cleared congeners (Chapter 5). Like all other groups (except the ones fed on spiked mayflies), accumulation of readily cleared congeners showed no significant difference between predicted and observed values. So this accumulation pattern was similar to what we had confirmed with the bioenergetics based model (Chapter 4) for wren nestlings - the nestlings showed very limited biotransformation ability. This may be because we looked through a very narrow window, a period of only seven days (Chapter 5). The slowly cleared congeners also confirmed the pattern observed with the bioenergetics based model.

The nestlings' final body weight at the end of the study differed significantly as a function of the type of mayfly supplement. The PCB spiked and Monroe, MI groups were significantly lighter than all of the other groups. The Monroe mayfly-fed group was

significantly different from all groups except spiked ones but the spiked group was similar to the ones fed with mayflies from the Point Pelee area, but different from other two groups. Final weight of the Monroe group was most noticeably different from all other groups. This was evident when the relative growth rates of different groups were compared. The other most abundant organochlorine, DDT, did not differ significantly among groups. So it is not clear whether the observed differences in growth are a consequence of the organic contaminants examined in this study or due to some other contaminant, such as metals that may be present especially in the Munroe group. A detailed analysis of all the contaminants including different metal loads in these different mayfly samples will give more information on the observed phenomenon.

Uniqueness of this study

The ecological assessment of birds exposed to pesticides and other persistent contaminants is accomplished by assessing suites of endpoints. Most toxicity assessments and evaluations of exposure effects in birds are performed as manipulative experiments under artificial conditions in the laboratory (reviewed by Mineau, 2005). Test results typically cannot be extrapolated to wild populations of birds because of the “inability to accurately interpret the ecological significance of laboratory test results” (Mineau, 2005). My research is significant in this context in that I have conducted a manipulative feeding experiment in a natural setting using field-collected rations to assess contaminant dynamics in avian populations. This study is unique in that the birds comprising the study treatments were exposed to all of the natural physical and biological interactions of their native habitat but were provided with food items collected from different locations that varied in susceptibility to environmental contamination. In order to augment the natural contaminant content, a spiked food treatment was also used, but it exhibited different exposure dynamics than the natural food-supplemented treatment groups.

Implications for health assessment of passerines in Point Pelee National Park

Passerine nestlings developing in the vicinity of Point Pelee National Park are exposed to persistent pollutants through their food and through the eggs. The maternal contribution from the egg becomes significant whereas the diet contribution is minimal.

When the egg contribution becomes a significant determinant, the feeding regime of maternal parent prior to oviposition becomes more of a concern. The bioenergetics and the growth rate also have very important roles in determining contaminant bioaccumulation. Biotransformation and contaminant elimination are also important; especially when contaminants are accumulated from breeding grounds, where the nestlings develop, in migratory passerines such as the three species investigated in this study.

The present levels of contaminant in the Park are not detrimental to the development and fledging of nestlings (Smits *et al.*, 2005; personal observation during study years) but if the contaminant load is augmented in some way it will be manifested as growth retardation of nestlings. Growth retardation and hepatomegaly were demonstrated in wren nestlings by the feeding experiments in which the nestlings were given mayflies collected from another more heavily contaminated region of Lake Erie (Corkum *et al.*, 1997). Mayflies can disperse relatively long distances during their landward flights (Kovats *et al.*, 1992; Corkum *et al.* 2006) so the experimentally induced effect can happen naturally in Point Pelee National Park especially in insectivorous birds that feed on mayflies. The effects become significant when the nestlings are exposed to more than one or to different kinds of POPs at specific concentrations (Chapter 5). We did not observe any cases of contaminant related reproductive impairment in any of the three study populations. Predation and inclement weather were more important factors that limited fledging success in the park, especially for tree swallows (personal observation).

Overall conclusions

Every field research project endeavours to study a system in detail. By that definition, I was able to study the contaminant exposure routes of nestlings of three insectivorous passerine species. If the goal of field-based studies is to find general patterns, my work was successful according to that definition. The three study species are members of the same guild (insectivorous passerines). However, each species exhibits unique foraging strategies and food collection niches. This is reflected in the significantly different relative proportions of aquatic and terrestrial insects in the diet of each species.

The differences in fledging weight among species are also large - purple martin biomass at fledging is double the biomass of tree swallow nestlings, which is double the biomass of house wrens. So the comparative study of these three species that are collectively classified as ‘insectivorous passerines’, is very interesting and informative. A study of tree swallow populations at various geographical locations will reveal variation in contaminant accumulation, but the proportion of diet (aquatic vs. terrestrial) of swallow populations will tend to be consistent among locations. Feeding experiments in the park with wren nestlings demonstrated how growth could become retarded and organs become hypertrophied as a consequence of toxic exposure from feeding on natural insect food from a contaminated habitat.

The results of the combined feeding study and the bioenergetics based estimation of Σ PCB and Σ DDTs can be used as a tool to estimate intra- and interspecific diet differences and hence the type and amount of contaminants accumulated. Because different individuals of the same species and individuals of different species track available resources differently at certain times, their diet composition will vary, and these differences can be tracked by analyzing differences in accumulation of biochemically stable compounds such as PCBs and DDT. In other words, these compounds can be used as ecological tracers to assess diet or other physiological processes in animals (Hebert *et al.*, 2009).

One of the major goals in ecotoxicology is to make accurate and cost-effective predictions of the movement of chemicals, including biological transfer and impacts, in the ecosystem starting with individual organisms, and progressing to populations and then communities (Cairns and Pratt, 1993); and those tests should be relevant, reliable and repeatable (Calow, 1992). In this respect, my study of three species of passerine nestlings, exploring the contaminant dynamics, determinants of contaminant accumulation and the model used in predicting the contaminants are all applicable to other avian populations and thus to the avian community at large.

Future directions and suggestions

My original research goal was to evaluate the effectiveness of nondestructive biomarkers (especially porphyrin in the excreta) as effects indicators of exposure to

organic contaminants. Because of certain limitations in field work, I couldn't pursue that topic with my current research. If one were to evaluate nondestructive biomarkers in a sentinel passerine species, it would be advisable to study a species of slightly larger size, longer fledging duration and to evaluate a slightly larger sample size to better distinguish different effects. It is very important to develop nondestructive biomarkers that cause minimum disturbance to individuals and thus to the population.

The feeding studies showed that natural food, which contains a mixture of contaminants, or other qualitative aspects of the food (lipid content of different mayfly groups) rather than PCBs affects overall nestling growth and enlargement of organs. Consequently, it is recommended to analyze the study mayflies for potential differences in the signature of other classes of contaminants (e.g., trace metals; PAHs). Ideally, one should conduct another experiment that involves feeding different bird species simultaneously to determine if same effect can be demonstrated among the study species. If another class of contaminants is found at potentially toxic concentrations in Monroe mayflies, another nestling feeding experiment, which uses that particular contaminant augmented in food should be conducted to determine how those chemicals influence in avian nestling health and development.

When insectivorous passerines that can accumulate relatively high concentrations of certain POPs without exhibiting severe impairments are used as sentinels, and biomarkers are developed based on their effects, it is doubtful that more sensitive species will be protected based on subsequent monitoring studies. Therefore, it is important to develop regulations and guidelines that protect the more sensitive species.

Migratory birds carry contaminants from their prebreeding locations in their tissues and pass them on to their nestlings. When those nestlings are used as sentinels, it is essential to have detailed background information on the pollution conditions of their maternal prebreeding locations. Accumulation from breeding grounds and overwintering locations can be studied by comparing the contaminant signatures of eggs from the first clutch with burdens of succeeding clutches and also by looking at the signature of eggs for readily cleared PCB congeners or other organochlorine contaminants like dieldrin (Clark *et al.*, (1987; Drouillard *et al.*, 2001; 2007).

Other areas of importance to study include the detoxification abilities, metabolic pathways and elimination capabilities of passerine nestlings. This research will help us to understand how each species tolerates persistent bioaccumulative pollutants and how a species' ability to biotransfer differs from other species that share the habitat. It is especially informative to undertake a lab study that feeds nestlings with diets of known contaminant concentration and to subsequently conduct depuration studies. A time series study of above will also provide information on nestling's absorption/assimilation efficiencies too.

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Appendix 1: Spatial, temporal, and dietary determinants of organic contaminants in nestling tree swallows in Point Pelee National Park, Ontario, Canada

Introduction

Across Canada localized areas have been contaminated with organic pollutants from the historic use of persistent industrial or agricultural chemicals¹. Point Pelee National Park (PPNP) in southwestern Ontario is one such area, with contamination from historically used chlorinated organochlorine pesticides (principally DDT and its degradation products and dieldrin) during the 1950s and 1960s^{2,3}. Point Pelee National Park is Canada's second smallest national park, consisting of 20 km² of wetlands, Carolinian forest, beach habitats and old fields (former farms and orchards) within a highly fragmented landscape. It is internationally known as an important staging area for migratory birds during spring and fall migration, and a vital breeding area for many species of birds, especially passerines.

This study was launched because of the recent discovery that highly localized areas within the park boundaries had high levels of persistent contaminants stemming from previous agricultural practices. Specifically, DDE, DDT and dieldrin exceeded the Canadian Council of Ministers of the Environment (CCME) guidelines, which are the conventional reference criteria for assessments of federally owned sites, as well as the provincial criteria as established by the Ministry of the Environment (MOE) for near surface soil samples². Because of the ubiquitous nature of this class of contaminants on a global scale, and because tree swallows are increasingly playing the role of sentinels for other avian wildlife exposed to environmental pollutants, the current investigation into risks to wildlife was launched.

The insectivorous tree swallow (*Tachycineta bicolor*) feeds primarily on flying insects. This species has been widely studied to document the biological effects of anthropogenically imposed environmental stress⁵⁻⁸. They are passerines that forage within approximately 100 m of their nest site during the breeding season^{9,10}. Tree swallows are effective integrative biomonitors of local organochlorine contamination because of their position high in the food web^{11,12}. Birds living in habitats with contaminated soils or sediments are thought to feed almost exclusively on insects whose immature stages have developed locally, and therefore would accumulate persistent compounds from these food

items¹¹. Female birds may pass contaminants stored in their body tissues on to their eggs and thus affect embryos during development^{12,13}. However, nestlings receive additional dietary contaminants while the parents raise them on locally captured prey items. Thus, offspring are variably exposed to any xenobiotic compounds that exist in, or have bioaccumulated through, the local food chain. The biomass of tree swallow nestlings increases by an order of magnitude between the time that they hatch and the time that they fledge 15-20 days later. Therefore, we expected that the body burden of contaminants would predominantly reflect their diet rather than maternally derived xenobiotic compounds.

The developmental history and ecological requirements of the larvae of insect prey greatly influence the types of contaminants that they may accumulate and pass on to predators^{14,15,16}. Adult terrestrial insects whose larval stages are aquatic tend not to feed as adults¹⁷. Thus, their entire body burden of contaminants is derived from the sediments, food, or water where the immatures developed. Insects that have terrestrial larvae may acquire different suites of contaminants during their development in soil or on plants, and additional persistent chemicals through their feeding and dispersal behaviour as adults.

Swallows are opportunistic feeders, catching the most abundant insects available at the times of day and season during which they are active^{18,19}. Aquatic insects often dominate the diet (midges, mayflies, caddisflies)^{8,10,11,12}, but terrestrial insects can be an important component of the diet during emergence periods^{19,20,21}. The purpose of this study was to determine whether tree swallows breeding in PPNP were being exposed to persistent organic contaminants known to exist in areas within the park, and to determine the degree to which exposure is explained by focal environmental contamination. The role of dietary composition in explaining contaminant transfer in nestling tree swallows was also investigated.

Materials and methods

Study sites

Within the boundaries of PPNP former orchards are contaminated with organochlorine pesticides (OCs) such as DDT, its degradation products (DDD, DDE) and dieldrin. Ten soil samples (top 5 cm of earth) were collected from four different locations in 2001 and analyzed for the presence of a suite of organochlorine pesticides

(principally DDE, DDT and dieldrin)². Concentrations of dieldrin, DDE and DDT exceeded Ontario Ministry of the Environment (MOE) guidelines (1996)² and the Canadian Council of Ministers of the Environment (CCME) Environmental Quality Guidelines²² on three sites. The two areas that we defined as ‘contaminated’ for our study were those at which OC levels exceeded MOE guidelines, (e.g., DDE up to 33.31 µg/g, (9 of 10 soil samples exceeded MOE) DDT up to 22.14 µg/g, (8 of 10 exceeded MOE) and dieldrin up to 1.56 µg/g) (7 of 10 exceeded MOE). One reference site (4-R) within 4 km of the park was at the administration building of PPNP, which is surrounded by a natural area of willow shrubs, forbs and grasses.

In March 2002, we erected a total of 50 standard nest boxes for tree swallows apportioned among two nominally contaminated sites and two reference sites, based upon the *a priori* classifications described above using a combination of the soil residue report² and the historic land use (i.e., orchards), because the soil samples were taken from point locations chosen to represent more extensive areas. All sites were within 100 m of small bodies of water and within 400 m of Lake Erie. The two contaminated sites (1-C: 600 X 300m; 2-C: 300 X 100m) were 1 km apart at the nearest points, one reference site (3-R 400 X 150m) was 400 m from the nearest contaminated site, and 4 km from the second reference site (4-R; 40 X 40m).

Boxes were placed in open areas with scattered shrubs and trees. Because there are few such areas within this forested park, our ‘reference’ study sites had to be established based upon availability rather than a confirmed absence of elevated soil OC residues. The two areas that we designated as reference, had a similar land use history as other locations around the park that had been shown to have lower OC levels in the surface soil (below detection limits of 0.004 ug/g) (Table 1). A total of 15 boxes on reference sites and 20 boxes on contaminated sites supported successful nests (those with breeding pairs of tree swallows that raised nestlings).

Dietary studies

To investigate the link between food items consumed by the nestlings and potential sources of persistent contaminants, dietary samples were collected from selected 7-10 day old individuals from each site. We placed collars on nestlings to prevent them

from swallowing the insects provided by their parents. Sampling took place in the early morning or evening when the air temperature was moderate (15 - 25° C), to minimize stress to the nestlings, and to correspond with times of maximal parental foraging activity. An elastic band was placed around the base of the throat of each nestling. The band was secured by sliding a 3mm section of a latex #8 French feeding tube (Benlan, Inc. Oakville, ON) along its length, tightening the band enough to prevent passage of the food bolus into the esophagus, but not enough to inhibit normal gaping behaviour and breathing (J. Smits, unpublished). Chicks were immediately returned to the nestbox. The provisioning behaviour of the parents was watched from a distance of 50-100 m. After 30-45 min, or after the parents had made four to six trips to feed the young, chicks were taken out and any food bolus was removed from their crops using a curved hemostat. The nest and environs within nest boxes were also examined for any boluses that may have been regurgitated. The boluses from all the chicks in one nest were pooled and preserved in a single jar with 95% ethanol.

In the laboratory, the boluses were gently teased apart with fine forceps beneath a dissection microscope, and the insects separated, identified and quantified. The insects were in good condition, so almost all could be identified. They were identified to at least the order level. Diptera were identified to family.

Diet per nest box was described by number and by biomass per insect taxon. Because ethanol preservation of the boluses resulted in loss of an indeterminate amount of body fluids, insect biomass could not be determined directly by weighing. Instead, the length of each insect was measured to the nearest 0.1 mm using an image analysis system. Lengths were converted to biomass (dry mass) according to conversion equations reported by Benke²³.

Contaminant analyses

Tree swallow collection and preparation:

Two nestlings from each nest were randomly selected at 12 days of age, when they reached maximum body mass (J. Smits, unpublished). They were anaesthetized with an inhalation anaesthetic (Halothane[®]; Halocarbon Laboratories, River Edge, NJ, USA), euthanized by exsanguination, and necropsy examinations were conducted. Stomach

contents, bursa of Fabricius, spleen, thyroid glands, plus a section of liver were removed and preserved for other analyses. The remainder of the carcass was skinned, wrapped in xylene-rinsed foil and frozen at -20°C for OC residue analyses.

Site	A priori designation (<i>n</i>)	Contaminant	Soil residue ($\mu\text{g/g dry wt}$)	2001 Tissue residue ($\mu\text{g/g wet wt}$) ^b	2002 Tissue residue (ng/g wet wt) ^b ; $0 \pm \text{SE}$
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In 2001, of 10 samples of swallow carcasses pooled by site, five were from DDT-contaminated sites and five were from nominal reference sites. Because there was poor concordance between tissue residue levels of persistent OC and the site designations (Table 1), in 2002 we analyzed 20 samples of nestlings from individual nests. Eight samples were from reference area nests and 12 were from contaminated area nests. Some of these nestlings had been used for the dietary collections, but there was incomplete concordance between nests sampled for tissue residues and those from which dietary samples had been collected.

Insect collection and preparation:

Several collection methods were used to acquire sufficient insect biomass for contaminant analysis. Modified Pennsylvania-style light traps²⁴ were operated for 2-h periods beginning at dusk on one evening during the times when parents were provisioning for their young in 2002 and 2003. Pairs of traps, operated at each of sites R1 and C1 collected large quantities of adults of aquatic insects but relatively little biomass of terrestrial insects except for Lepidoptera. Daytime sweep netting and hand-collecting individual terrestrial insects from vegetation was subsequently used to augment quantities of these taxa. Insects collections were frozen en mass in hexane-rinsed glass jars. In the laboratory, they were thawed, sorted into taxonomic groups, weighed, and stored refrozen in hexane-rinsed foil until analyzed. Sufficient biomass was collected to permit analysis of a sample of each of three taxa from each of the two sampling sites.

1. ^a ND = not detected; DDE = dichlorodiphenyldichloroethylene; NS = not sampled.					
2. ^b From pooled samples of 34 individual birds in the area.					
1-C	Contaminated (6)	ΣPCBs	ND	87	429 ± 97.4
		DDT	7.8	1	16 ± 7.7
		DDE	13.8	7	309 ± 111
		DDD	0.2	8	5 ± 1.0
		Dieldrin	0.5	3	18 ± 5.9
		Σ Other OCs	<0.5	4	13 ± 1.7
2-C	Contaminated (6)	ΣPCBs	ND	365	510 ± 41.6
		DDT	9.5	2	11 ± 2.4
		DDE	12.2	93	281 ± 48.3
		DDD	0.2	4	4 ± 1.1
		Dieldrin	0.5	14	9 ± 1.8
		Σ Other OCs	>0.5	20	12 ± 1.4
3-R	Reference (3)	ΣPCBs	NS	109	786 ± 294
		DDT	NS	1	1 ± 0.5
		DDE	NS	3	85 ± 17.3
		DDD	NS	1	3 ± 1.8
		Dieldrin	NS	3	6 ± 2.0
		Σ Other OCs	NS	2	15 ± 4.5
4-R	Reference (5)	ΣPCBs	NS	141	515 ± 103.7
		DDT	NS	1	4 ± 1.6
		DDE	NS	15	125 ± 22.5
		DDD	NS	7	3 ± 0.6
		Dieldrin	NS	5	9 ± 1.1
		Σ Other OCs	NS	5	17 ± 3.

Table 1. Residues of polychlorinated biphenyls (PCBs) and organochlorine (OC) pesticides in soil samples as well as in tissues of nestling tree swallows in 2001 and 2002 in Point Pelee National Park, Ontario, Canada^a

Analytical Procedures:

Contaminant analyses were performed by the Great Lakes Institute of Environmental Research Analytical Laboratory, University of Windsor, Windsor, ON according to standard analytical procedures using a Hewlett-Packard (Orangeville, ON, Canada) 5890

gas chromatograph equipped with an HP-5972 mass selective detector and HP-7673 autosampler²⁵. Tissues from nestling swallows and insect samples were analysed for 85 PCB congeners, DDT, DDE, DDD, dieldrin, cis-nonachlor, HCHs, mirex and others. Throughout this paper, “OC” will refer to the compounds listed above, excluding PCBs, which are presented separately.

Statistical Analysis

Contaminant concentrations in nestling tissues among sites were compared with one-way analysis of variance (ANOVA) of Log-transformed data. Planned comparisons were used to determine whether contaminant concentrations differed between the two reference and the two contaminated locations. Separate, Bonferroni-corrected univariate analyses were performed for the sum of DDT and its degradation products (Σ DDT), total PCBs (Σ PCB), and the sum of other organochlorine pesticides (Σ OCs).

Correlations between tissue concentrations of nestlings per nest box and the absolute or relative biomass of the dominant dietary items of aquatic and terrestrial origin were determined using Spearman’s rank correlation coefficient.

Results

Swallow Tissue residues

2001 samples:

Tissue body burdens of major OC and Σ PCBs in nestling tree swallows are presented from the four sites, C1 and C2 being the *a priori* labeled contaminated sites based on soil contaminant residues of OCs, while R1 and R2 are reference sites (Table 1). In 2001, nestlings from contaminated site C1 had OC and Σ PCB levels similar to those at the reference sites, which were lower than those in the other contaminated area (C2, Table 1). Polychlorinated biphenyls were detected in all pooled tissue samples of tree swallows, but were below detectable levels in the soil samples collected throughout the park².

2002 samples:

Tissue concentrations of persistent contaminants were determined from the nestlings of individual nests within the different sites. Tissue residues were, on average, much higher in 2002 than in 2001 (Table 1, Fig.1). In 2002, there was better agreement between soil and tissue OC levels than had been observed in 2001. Overall, the concentration of \sum DDT was significantly higher in the tree swallows collected from the contaminated areas than in nestlings from the reference areas (ANOVA $F_{1,18} = 11.31$, $p < 0.01$). Mean nestling concentration of \sum DDT varied significantly among sites ($F_{3,16} = 3.80$, $p < 0.05$; Fig. 1). Concentrations in nestlings from the two contaminated sites were significantly greater than concentrations in tissues from the reference sites (planned comparison, $F_{1,16} = 11.26$, $p < 0.005$). In birds from the two contaminated sites (C1 vs C2) and the two reference sites (R1 vs R2) respectively, tissue contaminants were not different from each other ($F_{1,16} = 0.10$ and 0.76). The pattern was the same for an analysis based upon the total concentrations of \sum DDTs plus dieldrin and other pesticides. In contrast, for PCB residues in tissues of nestlings, there was no difference between nests located in contaminated areas and those in reference areas ($F_{1,16} = 1.14.73$, $p = > 0.05$, planned comparison), or among the four sites ($F_{3,16} = 0.99$, $p = > 0.05$) (Fig 1b).

Diet

Aquatic insects in the diet were represented primarily by two aquatic taxa (Chironomidae (midges) and Ephemeroptera (mayflies, almost entirely *Hexagenia*)) and two terrestrial taxa (Formicidae (ants) and Asilidae (robber flies)). These four taxa typically (13 of 17 nest boxes) accounted for over 90 percent of the biomass of food boluses and they represented at least 68 percent of the diet collected from any nest box.

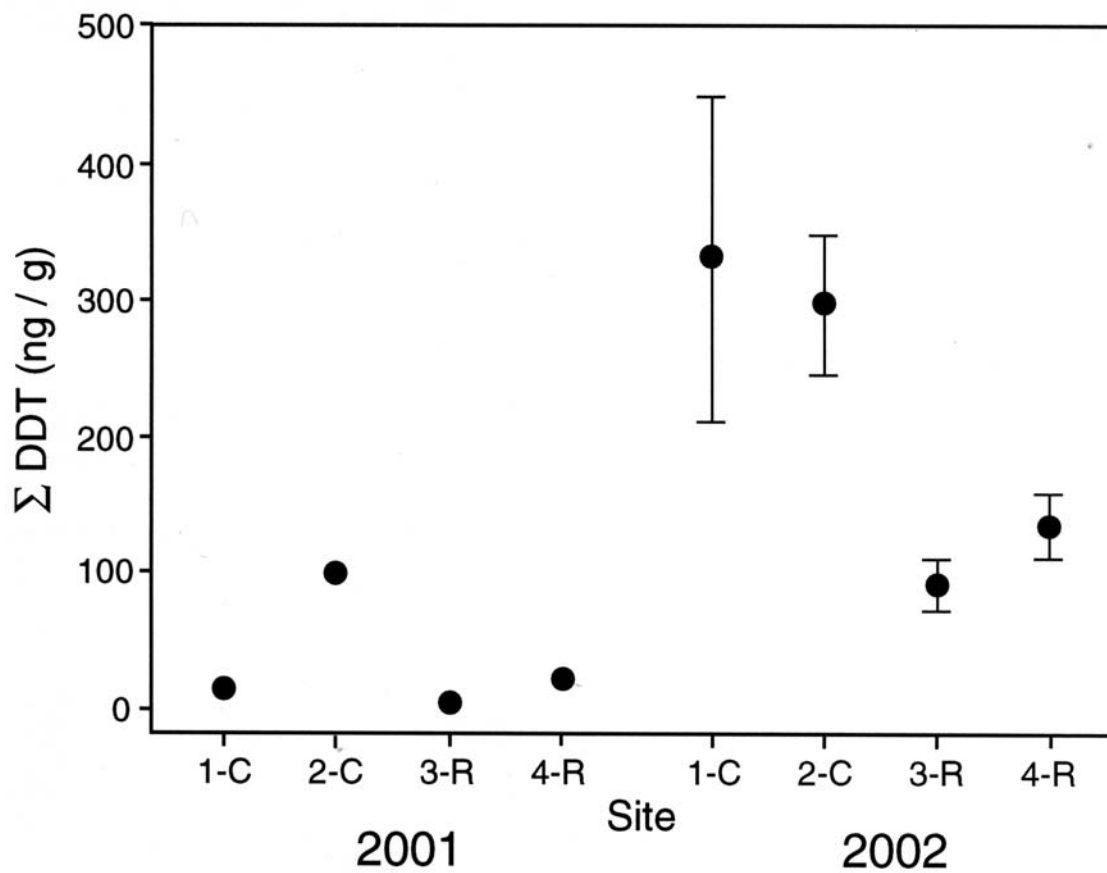


Figure 1. Tissue concentrations of DDT and its degradation products in 13 day old nestling tree swallows from contaminated (1-C, 2-C, based upon soil residue analysis), or reference (3-R, 4-R) sites in 2001 and 2002.

Chironomids were numerically most abundant, but *Hexagenia* made up most of the biomass (Table 2).

There was much more variation in diet from nest box to nest box than among sites. We observed considerable variation in tissue contaminant levels and dietary items of nestlings both within and among the sites, and between years. Consequently, the composition of the diet was investigated as a possible explanation. Insect prey items recovered from food boluses were classified as aquatic (having at least the larval stage spent in water – Chironomidae and *Hexagenia*), or terrestrial (all life stages spent on land). The few semi-aquatic invertebrates collected (those whose larvae develop in saturated soil) were included in the terrestrial category. The proportion of prey items of terrestrial origin in the diet of the nestlings (based upon biomass) was positively correlated with the nestlings' tissue residue levels of \sum DDT (Spearman rank order correlation, $r_s = 0.60$; $p < 0.05$; $n=13$). However, when the biomass (weight of insects delivered per nest) was considered, the association was weaker and not statistically significant ($r_s = 0.38$, $p > 0.05$, $n=13$). Almost identical correlations to \sum DDT were found between tissue concentrations of \sum OCs and the proportion of total biomass made up by the various insect groups ($r_s = 0.07$ (Chironomidae) $p > 0.05$, -0.45 (*Hexagenia*) $p > 0.05$, and 0.57 (terrestrial insects) $p < 0.05$). Tissue burdens of \sum PCBs were highly significantly correlated with the biomass of *Hexagenia* mayflies whether determined based on total weight ($r_s = 0.71$; $p < 0.01$; $n=13$) or as a percentage of the total biomass ($r_s = 0.82$; $p < 0.001$; $n=13$) (Fig. 2). Concentrations of \sum PCBs were not correlated with either absolute or relative biomass of Chironomidae ($r_s = 0.01$ and -0.04 , respectively; $p > 0.05$; $n=13$). The relative number and biomass of insects of terrestrial or aquatic origins were independent of the date that the prey were collected from the nestlings ($p > 0.2$). However, the biomass of mayflies, which was so strongly associated with PCB tissue levels, was highly negatively correlated with the date of clutch initiation for the nest (Pearson correlation, $r = -0.91$, $p < 0.001$, $n=17$) (Fig. 3).

Table 2. Geometric mean (SE) biomass (mg) of insects collected from boluses (*n* per site) of tree swallow nestlings in four areas of Point Pelee National Park, Ontario, Canada

	Biomass			
	3-R (<i>n</i> = 4)	4-R (<i>n</i> = 3)	1-C (<i>n</i> = 4)	2-C (<i>n</i> = 6)
Aquatic				
Chironomidae	60.7 (4.0)	122 (1.1)	25.9 (3.0)	11.6 (1.7)
Hexagenia	14.8 (5.0)	96.7 (1.9)	51.5 (6.6)	4.75 (2.3)
Terrestrial				
Asilidae	0.00 (0.00)	0.00 (0.00)	2.00 (3.00)	22.4 (2.1)
Formicidae	1.34 (1.91)	1.0 (2.0)	16.8 (3.1)	1.75 (1.78)
Other taxa	5.92 (1.32)	10.5 (2.3)	11.0 (2.5)	20.9 (1.3)

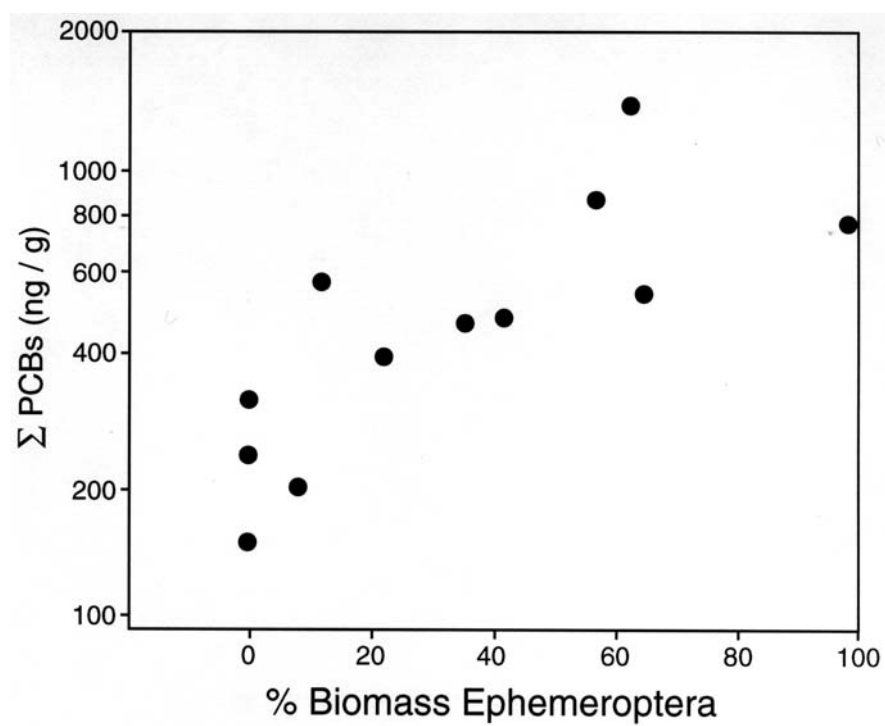


Figure 2. Tissue concentrations of Σ PCB in 13 day old nestling tree swallows in 2002, relative to the percentage of their diets comprised of ephemeroptera (mayflies).

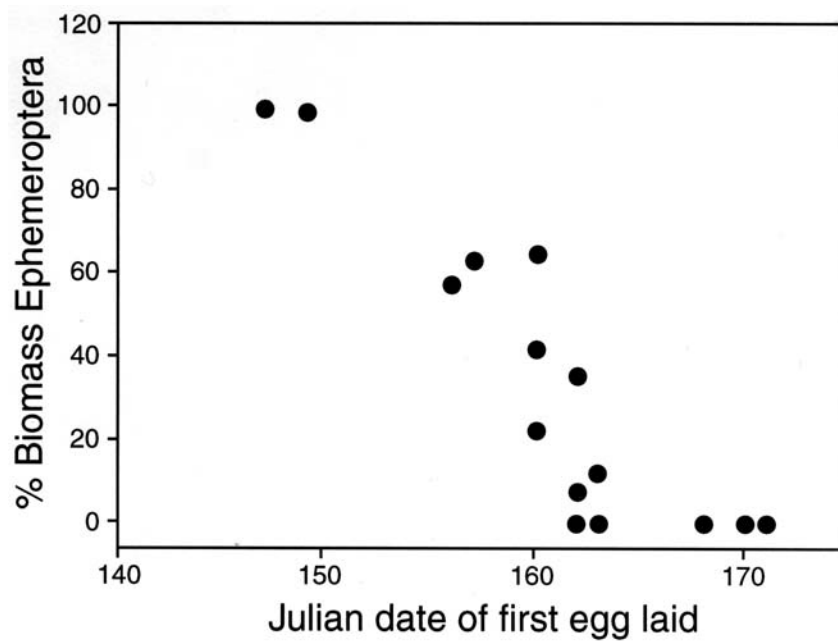


Figure 3. Percentage of mayflies in the diets of 13 day old nestling tree swallows relative to the date on which the first egg in their clutch was laid.

Discussion

In this study we examined whether persistent organic contaminants known to exist in areas within PPNP, were bioaccumulating in tree swallows breeding there. For the two years of this study, tree swallows were breeding throughout PPNP, regardless of the status of the soil contaminant levels. Since many of the OC compounds that are still being detected at unacceptably high levels in park soils are insecticides, one might expect this to have a detrimental effect on food supply of insectivorous birds. This ought to result in decreased numbers of breeding pairs choosing these sites for nesting. Furthermore, the bioaccumulative potential of chlorinated pesticides would lead to increased body burdens and possible negative biological effects in birds breeding on these sites. Our data present evidence that persistent environmental contaminants are accumulating in the nestling tree swallows, and that chlorinated pesticide burdens in particular are higher in nestlings raised in the contaminated areas than in those raised in reference areas.

Logic would argue that the presence of historically used pesticides in tissues of nestling birds reflects contamination of local food sources since tree swallows generally forage within 100 m of their nests^{8,9}. The levels of contaminants in viable embryos weighing 1.7 g, even without considering biotransformation, which is known to occur¹³, likely represents a minor proportion of contaminants found once the nestlings have reached their fledging weight of 22 g. Studies of black-crowned night herons (*Nycticorax nycticorax*)^{13,26}, reported that although concentrations of tissue contaminants decreased over time, the total amount of OC pesticides plus their metabolites in growing chicks was greater than that found in sibling embryos. In other words, the hatchlings continued to accumulate the contaminants throughout their nestling period through their diets. These observations support our rationale that OC accumulation in nestlings reflects local exposure.

Factors determining contaminant loads in birds are complex. The fact that diet, specifically the proportion of mayflies, was so variable even among boxes within the same local sites (Fig. 2), explains why we failed to observe site-scale differences in PCB contamination. The aerial insect prey of tree swallows are mobile and can be wind-dispersed. When insects from remote areas dominate the diet, local soil residues may

provide relatively weak information regarding risk to insectivores. We observed little site-specific consistency in tissue OC residues in nestlings between years. This may be an artifact of the different sampling methods used in the two years, (pooled vs nest-specific samples), or it might reflect other differences in the two years, most likely related to diet. In 2002, for which we had both nest-specific residue and dietary data, both the proportion of terrestrial insects in the diet and the insects' contaminant signatures were correlated with the types and amounts of contaminants in tree swallow nestlings, consistent with the bioaccumulation of local contaminants through the food web

Tissue concentrations of contaminants, especially PCBs, in the nestling tree swallows were more closely tied to the insect prey consumed at specific times during the nestling period than to local soil contamination conditions. The dietary data suggest that the parents of birds from different nests either foraged on different types of insects, or that there were dramatic differences in the types of insects available during the nestling period. In 2001, tree swallows reared most of their young during the first two weeks of June, and the nestlings had comparatively low levels of both PCBs and other OCs. In 2002, rearing of young was delayed by more than three weeks because of various weather- and predator-related reasons. This likely had a major influence on the major types of insect prey items available to the tree swallows, especially *Hexagenia* mayflies. Mass emergences of mayflies from western Lake Erie typically begin in late June²⁷, when the water temperature reaches 20 degrees C (J.J.H. Ciborowski, unpublished). Emergence reaches its peak 1-2 weeks following its onset and then gradually tapers off. The park is a thin peninsula extending approximately 10 km into Lake Erie thus being highly influenced by conditions of the lake. Adult mayfly activity (hence swallow foraging success) is inhibited by strong winds (especially from the north at PPNP), relatively low air temperatures and heavy precipitation^{28,29}. Although we lack detailed information, we expect that the emergence phenology of terrestrial species like ants would be seasonally regulated by the same weather conditions as limit the mayfly emergence. In 2001, the *Hexagenia* emergence period began in late May. Mass emergence did not begin until approximately June 28, 2002 (J.J.H. Ciborowski, pers. obs.) which followed the coldest month of May on record (Environment Canada, Atmospheric Environment Service, unpubl).

We have convincing evidence (Fig 2) that the nestling tree swallows with increased body burdens of PCBs were those for which mayflies dominated the diet. Samples of light-trapped *Hexagenia*, collected on the same date as food boluses in 2002 had PCB concentrations 6-10 times higher than locally-collected terrestrial insects (Formicidae). However, the concentrations of PCBs, dieldrin, and other organochlorine compounds were typical of adult *Hexagenia* emerging from western Lake Erie^{29,30}.

Food boluses were collected from 7-11 day-old nestlings over a two-week period. DDT levels in the nestlings were independent of the date they were euthanized, but consumption of terrestrial insects was related to higher tissue contamination with DDT. In 2002, the timing of clutch initiation, and thus the age of the nestlings corresponded with the period of peak availability of mayflies. Consequently, parents with the oldest nestlings (those hatching earliest in the season) fed their young proportionally more mayflies, which translated into disproportionately higher body burdens of PCBs.

Government agencies in Canada and the United States have derived tissue residue guidelines for the protection of human health and also wildlife. These guidelines differ somewhat among jurisdictions, but the level of total DDTs for humans and wildlife that consume aquatic organisms ranges from 0.14 to 1 mg/kg, with 0.4 mg/kg ww being considered protective²². Protective levels of Σ PCBs for aquatic and terrestrial wildlife are 0.1 mg/kg wet weight³¹. The birds analysed in this study had tissue residues well below those associated with reproductive impairment, (DDE ($\geq 4 \mu\text{g/g ww}$))¹ and PCB mixtures ($\geq 1\text{mg/kg ww}$)³² in a variety of other avian species for both although studies of individual congeners of PCB produced adverse effects at 10x lower concentrations. However, because the concentrations of PCBs in mayflies and DDE in ants in this study are above these guidelines, risks exist for birds consuming high proportions of these insects. Tree swallows are used to represent a wide range of insectivorous birds in contaminant related research^{4,5,8,12} even though, for reproductive endpoints at least, they are less sensitive to PCBs and other chemicals than are other insectivores^{18,33}. However, there is evidence that they may be more sensitive when considering other endpoints such as physiological or immunotoxicological variables^{8,34}. It would be valuable to study adult birds, or young of the year after they have spent longer periods feeding on

contaminated prey. Modelling studies could provide another means of determining contaminant behaviour in the insect-tree swallow food web in this area.

The organic contaminants found in the nestlings must have proximate sources. The DDT-related products appear to be derived from terrestrial insects emerging from areas fairly close to the boxes, which is indicated by higher tissue DDT levels in birds from boxes on contaminated sites versus those from reference sites in 2002. As well, these observations provide evidence of the spatial stability of DDTs in the soil of the park. Since PPNP is on a narrow peninsula where most wind effects would come from Lake Erie over the land, there is probably minimal drift of insects blowing major distances from the mainland to the study sites.

Our findings emphasize the importance and value of dietary details in explaining variability in burdens of bioaccumulative compounds. The study was designed to examine possible effects of OC contaminants, PCBs being undetected in the soil. Had the analysis relied upon comparisons of contaminated versus reference sites, the study would have concluded that there were no site differences in PCB contamination of nestlings, and that OCs were related in an unpredictable manner to soil contamination. Our detailed dietary examination elucidated possible food web routes for \sum DDT from soil to tree swallows, and identified the source of body burdens of PCBs which was independent of the *a priori* designations of the study sites. The localized OC contamination that was the recognized concern in the park, is not the only relevant contaminant problem for local insectivorous birds. The biological consequences of increasing tissue levels of OC and PCB must be evaluated before pollutant based risk are known. Trophodynamic studies such as this are a critical complement to risk assessment studies of natural populations.

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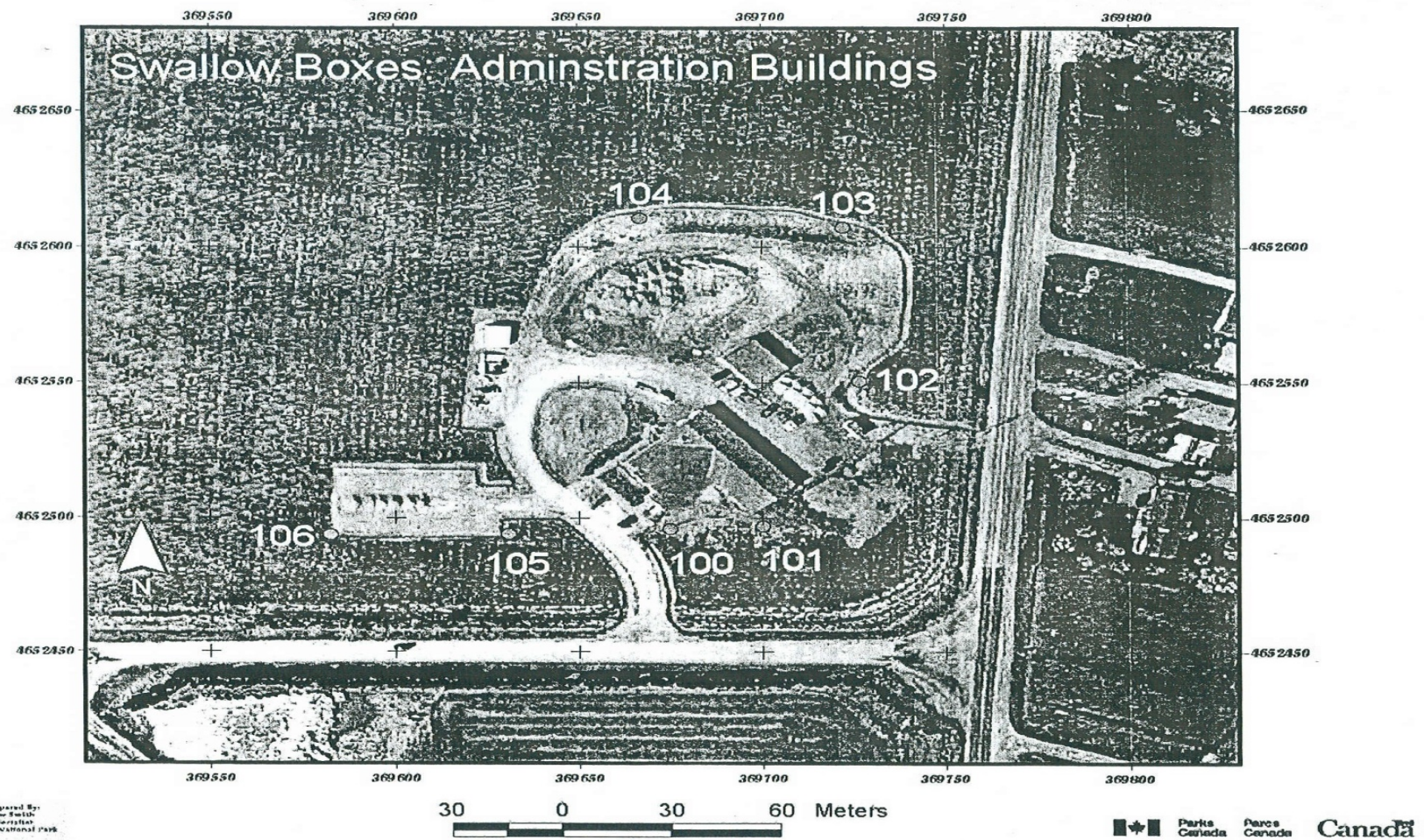
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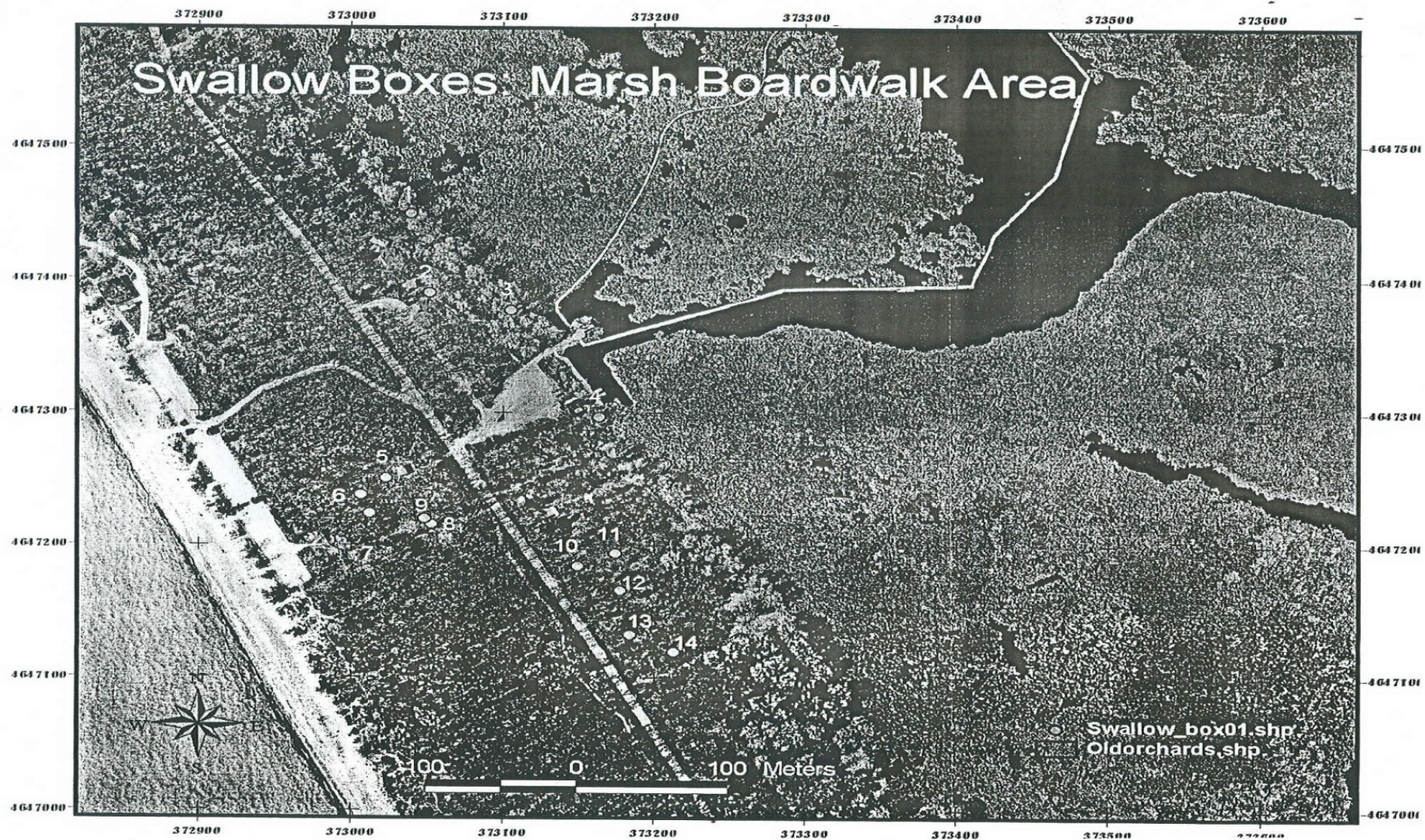
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Appendix Table 1. Data sheet showing the nest occupancy and breeding activities in 21 nest boxes in Marsh Board walk area of Point Pelee National Park on May 25, 2005

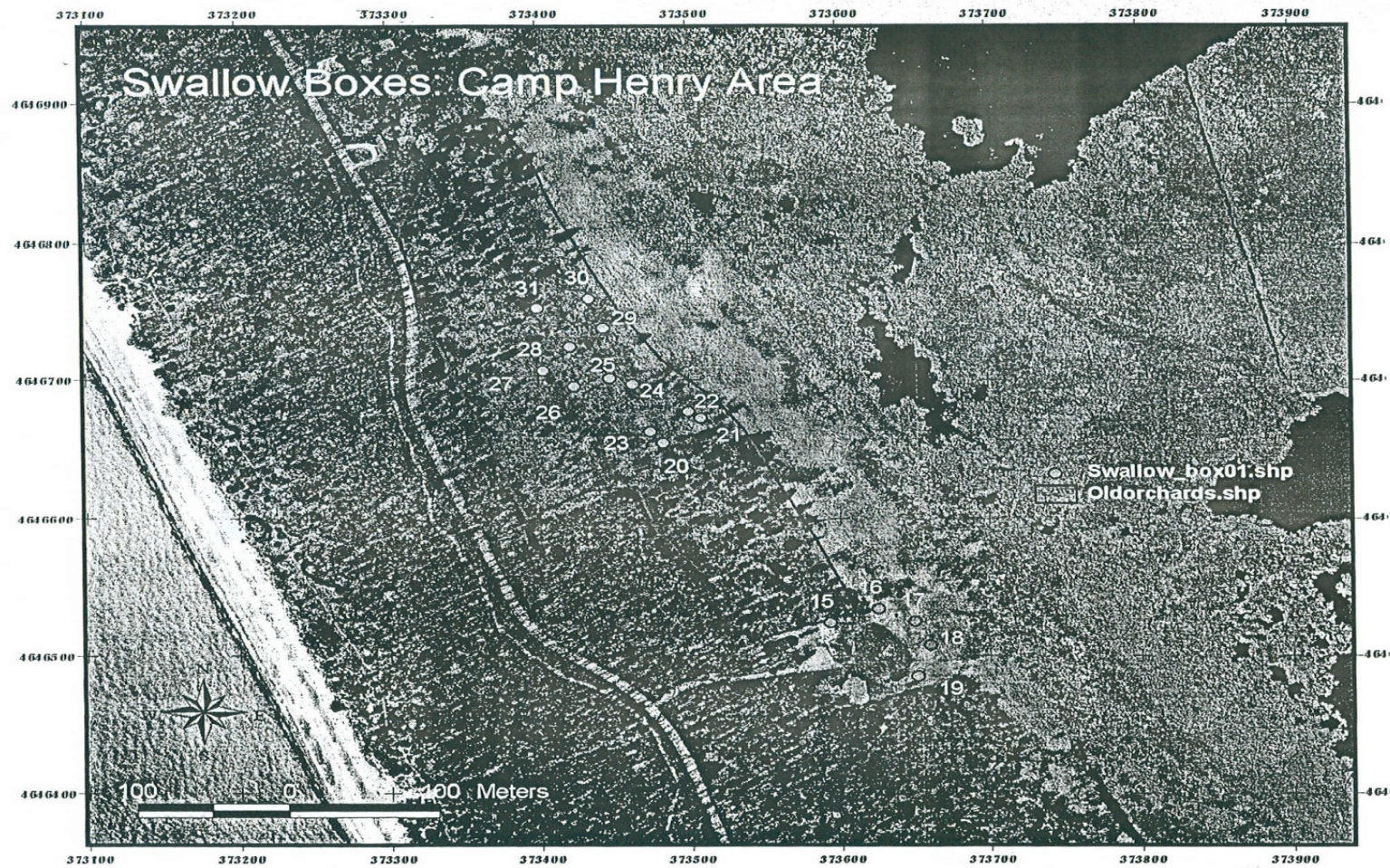
Nest #	Breeding activities	Eggs	Nestlings	Feathers lining the nest cup	Comments
1	Nesting activities				Nest materials twigs but swallows using
2	”	1	0	3	Clutch initiation day
3	”	1	0	5	Clutch initiation day
4	”	3	0	2	Clutch initiation day, May 22
5	”	7	0	10	Started incubation
6	”	0	0	1	Parents around
7	”	3	0	13	Parents aggressively guarding’ clutch initiation day, May 22
8	”	0	0	0	Nest building with grass
9	”	0	0	0	Nest building with grass
10	”	5	0	3	Clutch initiation day, May 20
11	”	4	0	3	Parents around, clutch initiation day, May 21
12	”	0	0	5	Parents around building
13	”	1	0	7	Clutch initiation day
14	Wrens nest				
15	Swallow nest	1	0	0	Clutch initiation day
16	”	0	0	0	Nest building with grass
17	Wrens nest				
18	Empty				
19	Swallow nest	0	0	1	Nest building activity
20	”	3	0	6	Clutch initiation day, May 22
21	No nesting activity				



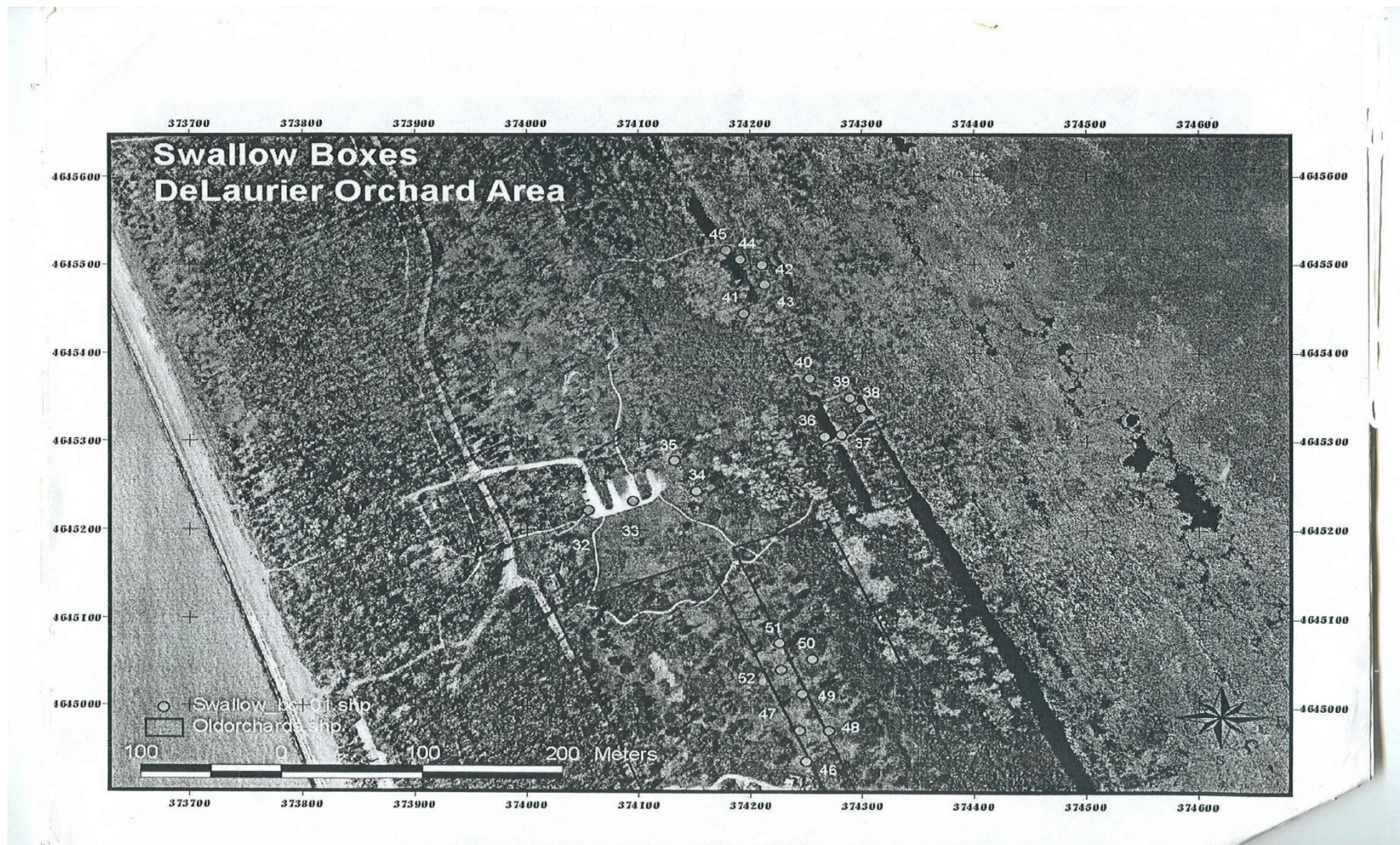
Appendix Figure:1. Swallow boxes in the administrative building area. Source: Point Pelee National Park, GIS department



Appendix Figure 2. Swallow boxes in the Marsh Boardwalk are Point Pelee National Park. Source: Point Pelee National Park, GIS department



Appendix Figure 3. Swallow boxes in Camp Henry area, Point Pelee National Park. Source: Point Pelee National Park, GIS department



Appendix Figure 4. Swallow boxes in DeLaurier Orchard area, Point Pelee National Park. Source: Point Pelee National Park, GIS department.



Appendix Figure 5. Placement of nest boxes in the marsh boardwalk area of Point Pelee National Park (source aerial map-Google earth)

Appendix 2: Diet of tree swallow nestlings developing in point pelee national park, Canada, determined by ligature and stable isotope methods

Introduction

Tree swallow (*Tachycineta bicolor*) nestlings are increasingly used as sentinel organisms for the accumulation of persistent pollutants in many ecotoxicological studies (Secord *et al.* 1999; Chu *et al.* 2003; Nichols *et al.*, 2004; Brasso and Cristol, 2008; Jayaraman *et al.*, 2009). While feeding their nestlings the parents collect aerial insects from a limited area surrounding their nests. This makes them as especially suitable bioindicators for localized contamination studies (Bishop *et al.* 1995, 1999). Tree swallows forage on insects of both aquatic and terrestrial origin while they are flying (McCarthy and Winkler 1999). Thus their utility as bioindicators depends upon determination of their diet before they are assigned for specific studies. Because tree swallows readily accept nest boxes, colonies can be established easily in any area of interest by providing suitable boxes (Bishop *et al.* 1995, 1999, Roof and Harris 2000). Previous studies suggest that aquatic insects, especially dipterans, are a preferred dietary component. Consequently, tree swallows tend to live and forage near water, and their prey of aquatic origin serve to transfer aquatic sediment-bound pollutants to the terrestrial ecosystem (Bishop *et al.* 1995, 1999, Nichols *et al.* 1995, 2004, Custer *et al.* 1998, 2000)

The ligature method is commonly used to study the diet of altricial nestlings. In this method, constrictive ligatures are placed around the neck of nestlings below the crop, which prevents them from swallowing the food provided by the parents (Johnson *et al.* 1980). The food trapped in the throat can be collected and can be identified. Advantages of this method are that multiple samples can be collected from one nest box at a time, and the same nestling can be sampled repeatedly. The bolus provided by the parents is well-coated with their saliva, which means that the entire mass of insects provided can be easily collected. Furthermore, the insects are mostly intact and can be easily enumerated and identified. However, ligature collections give information only on the diet consumed at that particular time. Consequently, use of some integrated measure of diet such as the stable isotope method is necessary to corroborate that the composition of a nestling's

food throughout the nestling period is accurately summarized by examination of boluses collected by the ligature method.

Stable isotope signatures are commonly used to provide information about feeding relations of an organism in a food web (Peterson and Fry 1987), to allow identification of multiple food sources consumed by an organism (Kucklick et al. 1996; Rush *et al.*, 2010; Ikeda *et al.*, 2010), to assess diet differences among groups of organisms of same species (Sanpera *et al.*, 2007) and also to evaluate dietary shifts in the same organism (Morrissey *et al.*, 2009). The ratio of heavier to lighter stable isotopes of nitrogen ($^{15}\text{N}/^{14}\text{N}$) expressed as $\delta^{15}\text{N}$, generally increases with trophic position in aquatic food chains (Fisk *et al.*, 2001). ^{14}N is selectively eliminated by organisms and ^{15}N incorporated in body tissues, so with each successive trophic level, $\delta^{15}\text{N}$, ($^{14}\text{N}:^{15}\text{N}$) values in the tissues of biota increase (Campbell *et al.*, 2000). Fresh water studies by Peterson and Fry (1987) indicate that the average $\delta^{15}\text{N}$ difference between trophic levels is between 3.2 and 3.4‰. Marine food web studies by Hobson *et al.*, (2002) have revealed a general pattern of enrichment in ^{15}N with trophic level and they suggests a 2.4‰ trophic enrichment for $\delta^{15}\text{N}$ between birds and their diets and 3.8‰ increase for all other components in the food web. An average value of about 3‰ is suggested by Minagava and Wada (1984). Nitrogen fractionation value for muscle tissue was determined by Hobson and Clark (1992) for captive juveniles of chicken, quail and gull. They found that fractionation changes quite small compared to previous studies - 0.2 ± 0.2 for chickens, 1 ± 0.1 for quails and 1.4 ± 0.1 for gulls.

The stable carbon isotope values do not fluctuate as carbon progresses through the food chain. There is less than 1‰ enrichment in $\delta^{13}\text{C}$ ($^{13}\text{C}:^{12}\text{C}$) per trophic level (DeNiro and Epstein 1978; Peterson and Fry 1987) but it would be highly variable at the base of the food chain or at the source of carbon. Hobson and Clark (1992) assessed carbon fractionation values also for muscles for three groups of juvenile birds they used to assess N fractionation values and found that chickens showed a value of 0.3 ± 0.3 and quails a value of 1.1 ± 0.5 and gulls 0.3 ± 0.4 . The trophic position of an organism and its primary carbon source can be assessed by determining the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in their tissues. However, stable isotope values cannot be used to distinguish the detailed

composition of terrestrial insects and aquatic insects in the diet. Therefore in this study we used a combination of the two methods.

The objectives of this study were to determine the diet of nestling tree swallows developing in Point Pelee National Park by both the ligature and stable isotope methods. The results of the study are used to answer questions of selectivity - are tree swallows feeding on what is available in the vicinity of the nest boxes? Does the composition of prey items vary through the nestling period? Even though many have studied nestling diet composition by different methods, this is the first study to combine stable isotopes and ligature methods to assess tree swallow nestling diets.

Materials and methods

This study was conducted in Point Pelee National Park. Point Pelee is at the most southerly tip of the Canadian mainland. It is one of the smallest National parks in Canada, but its landscape is a mix of marshes, forests, fields, and beaches. This makes the park habitats suitable for many life forms, breeding ground for many migratory and resident birds, and an important staging area for spring and fall migrations of birds. The park is situated in an area of overlap of the Atlantic and Mississippi flyways, two important continental routes along which many birds pass on migrations (Proctor and Lynch 1993; Fletcher, 1997). In spring, many birds stop temporarily in the park after the long flight northward across Lake Erie. Tree swallows and many other passerine species use the park for breeding (Stewart 1977).

In March 2001, a total of 50 standard nest boxes were erected to attract tree swallows in Point Pelee National Park (Appendix Figures 1-4). The nest boxes were placed in groups in four sites. (This project was designed to study the biomagnification of organochlorine pollutants, so details of the sites and others are described elsewhere (Smits *et al.*, 2005; Papp *et al.*, 2007)). The sites were named the Administrative Building Area (AB), Marsh Boardwalk Area (MB), Old Camp Henry Area (CH) and DeLaurier Orchard Area (DL). All sites were within 100 m of small bodies of water and within 400 m of Lake Erie. As far possible the nest boxes were placed in open areas with scattered shrubs and trees. The diet studies were conducted in June and July, 2002. The boxes were monitored for occupancy by birds and for breeding activities. A total of 35

boxes supported successful nests (those with breeding pairs of tree swallows that raised nestlings).

Ligature methods

The young are fed from the day of hatching, and both parents share this duty equally. When the chicks are around 7 days old (optimum size to handle, and engaged in adequate feeding activities), the young birds were collared around the neck just below the crop for up to 45 min. This prevents them from swallowing the food. Sampling took place in the early morning or evening when the air temperature was moderate (15 - 25° C), to minimize stress to the nestlings, and to correspond with times of maximal parental foraging activity. An elastic band or collar was placed around the base of the throat of each nestling. The band was secured by sliding a 3-mm long section of a 3 mm inner diameter latex tube along the length of the band, tightening the band enough to prevent passage of the food bolus into the esophagus, but not enough to inhibit normal gaping behaviour and breathing (Smits *et al.*, 2005). We briefly took individual nestlings out of the nest box to attach the collars. The tubing was kept on the dorsal side of the head, and the loop was tightened by carefully sliding the tubing towards the neck without exerting any pressure on the neck or any part of the nestling's body. We ensured that the rubber band collar was not tight enough to choke the bird or loose enough to allow it to swallow the bolus of food. After the collar had been fixed, the nestlings were placed back in the nest cup.

The feeding activities of the parents were observed from a distance. After around 45 min. or after the parents had made about 5-6 feeding trips, we collected the bolus samples from individual nestlings using a curved hemostat. The nest cups and the surroundings within nest boxes were also examined for any boluses that may have been spit out. The boluses from all the chicks in one nest were pooled and preserved in a single jar with 95% ethanol as a single, composite sample. In all, we collected 19 samples from among the four locations, which included samples from four age groups and from six different dates.

In the laboratory, the bolus samples were emptied into petri plates and the individual insects were separated gently with fine forceps beneath a dissection

microscope, and all organisms were identified and enumerated. Almost all of the insects could be identified to order, and the Diptera were identified to family. Identifications were mainly based on the keys of Borror, Triplehorn and Johnson (1992), the field guide of Borror and White (1970), and the Manual of Nearctic Diptera (McAlpine *et al.*, 1987). Insect prey items recovered from food boluses were classified as aquatic (having at least the larval stage spent in water – Chironomidae and *Hexagenia*), or terrestrial (all life stages spent on land). The few semi-aquatic invertebrates collected (those whose larvae develop in water-saturated soil) were included in the terrestrial category (Chapter 3).

Preservation alters the mass of invertebrates (Mills *et al.*, 1982; Lasenby, *et al.*, 1994; Johnston and Cunjack 1999). As biomass measurements were desirable, we estimated biomass from the relationship between body length and weight (Hawkins *et al.*, 1997; Benke *et al.*, 1999, Sabo *et al.*, 2002). The lengths of insects were measured to the nearest 0.1 mm from the anterior of the labrum to the posterior of the last abdominal segment (Smock 1980) using an image analysis system. Lengths were converted to biomass (dry mass) according to conversion equations derived by Benke (1996).

Stable isotope methods

One to two 12-day-old nestlings from each nest was randomly selected for stable isotope analysis. We collected tissues from 16 nest boxes to a total of 22 individuals. Two nestlings from a box were used to see variability within boxes. Nestlings were anaesthetized with an inhalation anaesthetic (Halothane[®]; Halocarbon Laboratories, River Edge, NJ, USA), and euthanized by exsanguination. The breast muscles were separated from the rest of the carcasses and stored in -20⁰ C for stable isotope analysis. The rest of the carcasses were used for other analyses.

All instruments used for tissue preparation were washed with soap and water and then rinsed with acetone. Tissues were dried in a vacuum drier for 16-24 h and then transferred from their drying tube into a pestle. The dried material was then ground using a mortar and pestle for about 5 min or until the tissues became a homogeneous fine powder. The fine powder was transferred to a scintillation vial, which was sealed with laboratory film (Parafilm “M”) to keep it airtight.

After determining the major insects in the diet, we collected them from the sites of nest boxes the following year and determined their stable carbon isotope signatures. Various sampling techniques including light trapping (Kovats and Ciborowski 1987), sweeping (both aerial and bush) and hand picking were needed to collect the different insect groups. The same techniques used for bird tissue preparation were used for insects' tissue preparations.

A Thermo-Finnigan Flash 1112 Series elemental analyzer was used to determine C and N isotope ratios of all above tissues (Stable Isotope Laboratory, Department of Earth and Environmental Sciences, University of Windsor). We used ants and robber flies (terrestrial) and mayflies and midges (aquatic) as carbon and nitrogen signature baseline organisms for the transfer of carbon and nitrogen for tree swallow nestlings. We estimated the relative contribution of each prey item to the diet of nestlings by using a mixing model run in the program MixSIR (this is a graphic interphase program (G UI) incorporated in MATLAB platform using sampling-importance-resampling(SIR) technique) (Semmens and Moore 2008). This program is based on Bayesian analysis. We used the isotope enrichment factors for muscles for birds provided by Hobson and Clark (1972). Their experiments used fast growing juveniles.

Data analysis

The mean proportions (by biomass) of aquatic and terrestrial insects comprising the diet of tree swallow nestlings derived from the two diet study methods, ligature and stable isotope methods were compared with a paired-comparison two-tailed t test. Additional analyses were performed to determine if there were differences in the proportion of food of aquatic origin that nestlings were eating (determined by the ligature method) at different ages, on different dates, and among the locations of feeding with the results obtained from ligature method. Two-way ANOVA was used to compare the estimates of the ligature catches with results of stable isotope method. Statistical program Statistica[®] version 6 software (Statsoft, Inc., 1997) was used for all analysis. The p level was set at 0.05 for all the analyses. As the values were in percentages they were arcsine converted before analysis.

Results

A total of 1,098 insects belonging to seven insect orders were counted and identified from 19 pooled bolus samples. The order Diptera was represented by 11 families of which Chironomidae represented 34% of the total biomass and Asilidae 9.4% and Ephemeroptera (*Hexagenia*) that contributed 43.3% and Hymenoptera that contributed 7.02% were assessed as the major groups of insects in the diet (Table 1). Insects of aquatic origin made up $72.5 \pm 32\%$ ($n=19$) of the insect biomass estimated from the ligature method (detailed composition of insects in bolus samples are given in Chapter 3).

There was no significant variation in the relative proportion of biomass of terrestrial and aquatic sources among nestlings of different ages ($F = (3,15) 0.59$, $p > 0.05$) (% mean \pm SE, 7 days old, 63.67 ± 11.41 , $n=9$; 8 days old 79.33 ± 19.77 , $n=3$; 10 days old, 77.93 ± 13.98 , $n=6$ and one 11 days old with an aquatic biomass 98.81%) or among collection dates ($F = (5,13) 1.9$, $p > 0.05$) from ligature methods (Fig. 1 and 2). But significant differences in the % biomass of aquatic and terrestrial insects eaten at different locations (% mean \pm SE, AB, 89.81 ± 14.11 , $n=3$; MB, 96.69 ± 10.93 , $n=5$; CH, 54.97 ± 9.97 , $n=6$ and DL, 78.17 ± 10.93 , $n=5$) as estimated by the ligature method ($F = (3,15) 6.23$, $p < 0.01$) (Fig. 3).

The stable isotope mixing model did not provide interpretable results. Evidently, as the diets included other food sources whose signatures were not known. There were great variations in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope signatures even within the 4 insect food types (diet insects) analyzed (Figure 4). The two ant samples analyzed exhibited a range equivalent to one trophic level's difference (1.89‰). The variation in nitrogen signatures observed between Asilidae samples was greater than one trophic level (2.59‰). Incorporating the same type of prey item itself could create an anomaly in the isotope signature of nestlings. We also had noticed variability in isotope signatures in nestlings also (Figure 5). A plot of isotope signatures of birds relative to the four types of prey insects shows that the nestlings' signatures fall outside the range of prey signatures (Figure 4). The bird signatures should fall within the boundaries of a polygon formed by

Table 1. Major insect taxa that contributed to the biomass of diet insects identified in the bolus samples of swallow nestlings by ligature method from 19 pooled bolus samples (* 1= administrative building area; 2= beach marsh boardwalk area; 3= camp Henry area; 4= DeLaurier area)

Box No.	Location of nest box in the park*	Date of Collection	Age of Nestling(days)	Chironomidae mass (mg)	Ephemeroptera mass (mg)	Asilidae mass (mg)	Hymenoptera mass (mg)	Dolichopodidae mass (mg)	Other diptera orders	Other insects
1	1	29-Jun	7	111.80	194.55	0	0	1.81	0.44	0
2	1	09-Jul	10	135.22	27.77	0	6.82	33.24	11.52	13.83
3	1	29-Jun	10	116.11	162.75	0	0	0	0	3.42
4	2	08-Jul	10	197.54	0	0	0.94	3.18	2.46	0.66
5	2	03-Jul	8	1.96	57.56	0	0	0	0.4	0.66
6	2	02-Jul	7	190.78	157.68	0	13.89	12.82	0.44	0
7	2	13-Jul	8	386.07	0	0	0	0	8.6	2.69
8	2	26-Jun	11	0	411.93	0	0	0	4.98	0
9	3	04-Jul	7	66.15	77.58	46.43	0.63	23.76	2.57	2.05
10	3	04-Jul	7	8.09	7.85	23.42	21.56	2.97	14.94	19.99
11	3	05-Jul	8	25.54	0	7.49	11.76	2.33	2.19	10.66
12	3	05-Jul	7	27.29	49.83	0	0	0	10.53	0
13	3	07-Jul	10	1.45	0	143.9	0	0	0.87	3.1
14	3	08-Jul	7	2.40	30.54	115.25	0	0	12.96	0
15	4	09-Jul	10	0.00	315.41	0	0	0	0	0

Table 1. Major insect taxa that contributed to the biomass of diet insects identified in the bolus samples of swallow nestlings by ligature method from 19 pooled bolus samples (* 1= administrative building area; 2= beach marsh boardwalk area; 3= camp Henry area; 4= DeLaurier area) (contd.)

Box No.	Location of nest box in the park*	Date of Collection	Age of Nestling(days)	Chironomidae mass (mg)	Ephemeroptera mass (mg)	Asilidae mass (mg)	Hymenoptera mass (mg)	Dolichopodidae mass (mg)	Other diptera orders	Other insects
16	4	10-Jul	7	84.63	0	0	11.2	0	22.01	11.6
17	4	11-Jul	7	98.12	115.94	84.17	206.87	9.45	11.45	3.47
18	4	12-Jul	7	60.21	206.24	0	39.01	2.38	5.37	0
19	4	13-Jul	10	0.00	112.4	0	0	0	0	0
		Sum		1513.36	1928.03	420.66	312.68	91.94	111.73	72.13
# of boluses the insects are represented				16	14	6	9	9	16	11
taxa %				34.00	43.32	9.45	7.04	2.07	2.51	1.62
Mean				79.65	101.48	22.14	16.46	4.84	5.88	3.80
SE				95.82	113.54	42.63	45.96	8.92	6.24	5.67

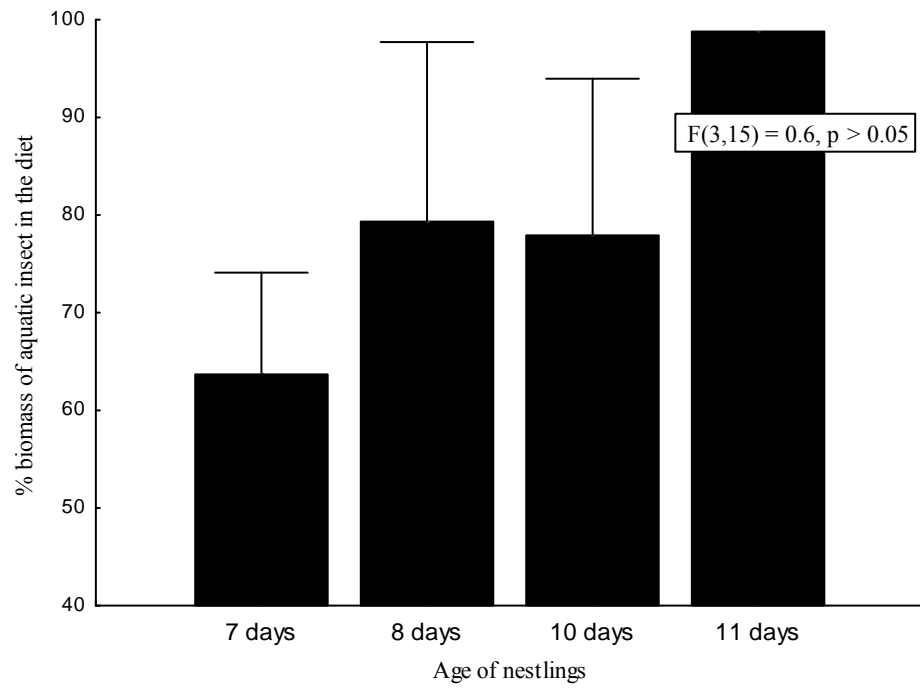


Figure 1. (Mean± SE) % biomass (g) of aquatic insects in the diet collected from of tree swallows nestlings of different ages by ligature method

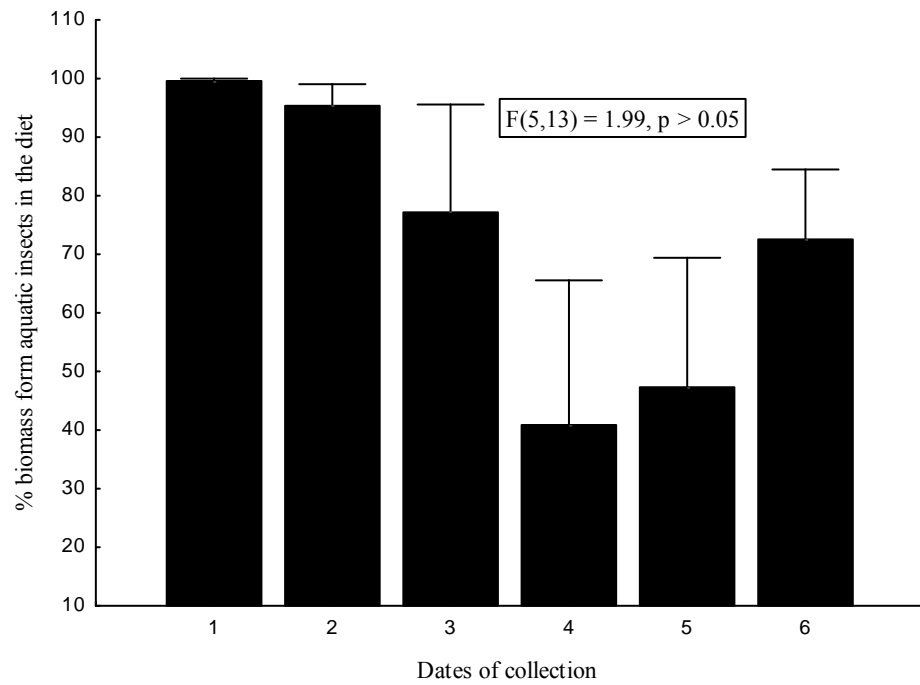


Figure 2. (Mean \pm SE) % biomass (g) of aquatic insects in the diet of tree swallows collected on different dates from June 26 to July 30, 2002 by ligature method

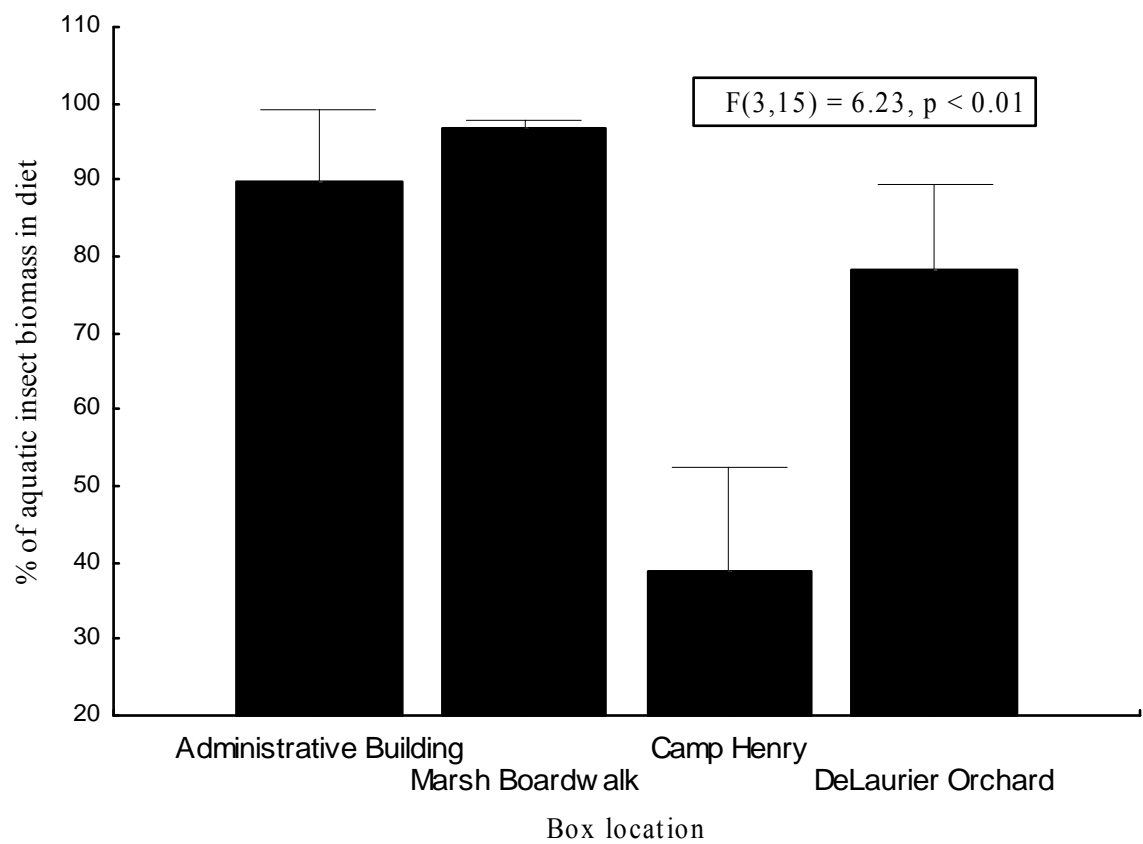


Figure 3. (Mean \pm SE) % biomass (g) of aquatic insects in the diet of tree swallow nestlings from ligature method from different locations

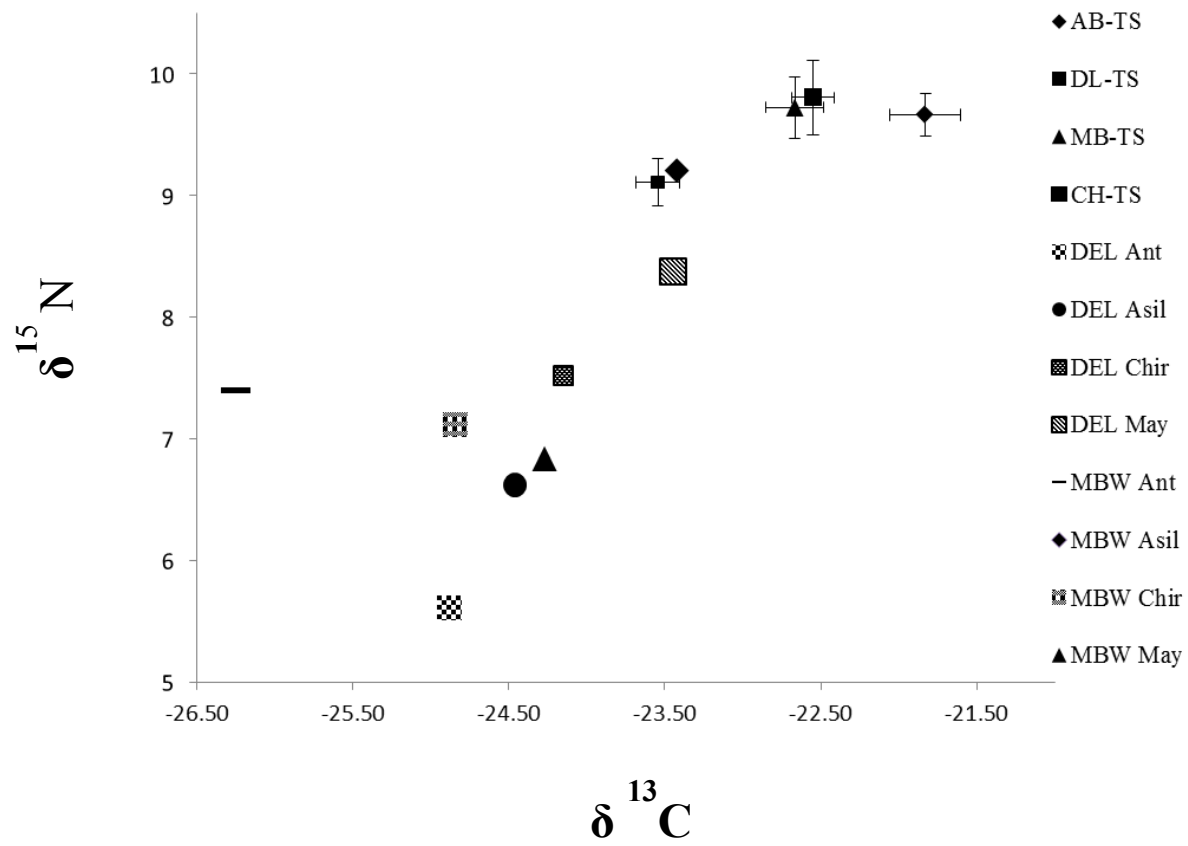


Figure 4. (Mean \pm SE) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of tree swallow nestlings from 4 locations TS= tree swallow nestlings (AB =administrative Building; MB=Marshboardwalk; DL=DeLaurier and CH=Camp Henry and insects ants, Asilidae (Asil), Chironomids (Chir) and mayflies (May) and from Marshboardwalk (MBW) and DeLaurier (DL)

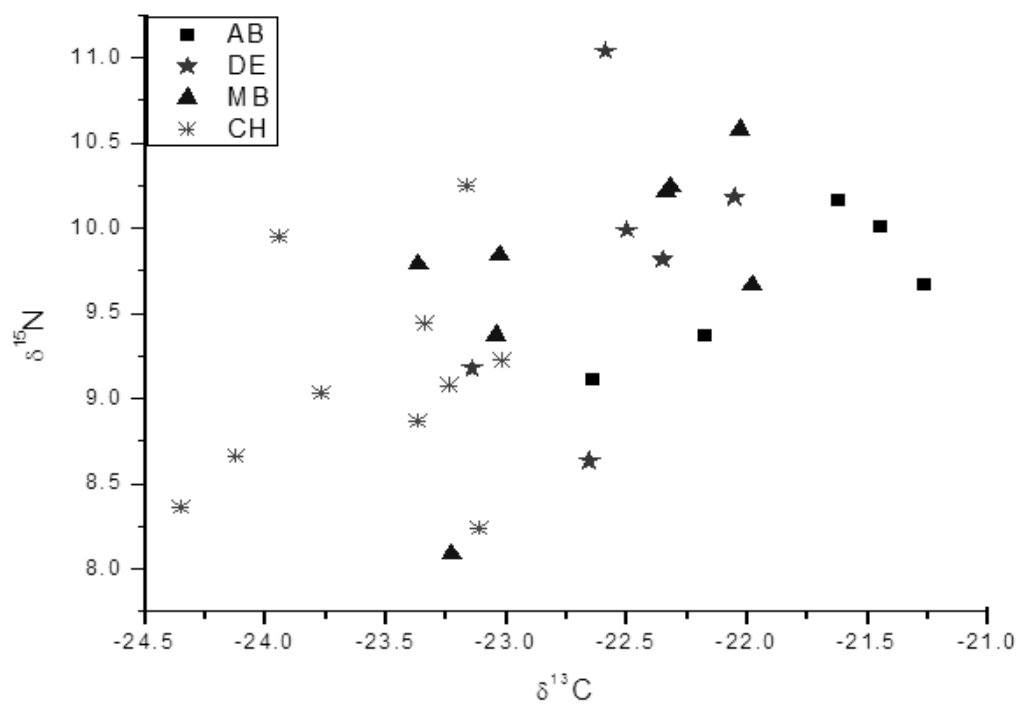


Figure 5. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of tree swallow nestlings from 4 locations in the park (AB= Administrative Building; DE=Delaurier; MB =Marsh Boardwalk and CH= Camp Henry area)

lines connecting the points representing signatures of each of the four prey types. Mixture model equations can only be solved if there are food items whose range of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values exceed those of the nestlings (Philips, 2001; 2002).

Discussion

The results of the ligature method indicate that tree swallow parents feed their nestlings with almost the same composition of insects throughout the nestling period, and with whatever insects are available locally. This was evident from the similarity in the diet composition on different dates of collection and from different age groups. The same results were obtained in studies conducted by Mengelkoch (2004). Previous studies determined that Diptera were the most preferred insect group (McCarty and Winkler 1991; Johnson and Lombardo 2000) in the diet. Our observations confirmed this, but we found that Ephemeroptera were almost as important a food source. At the family level of classification, Ephemeridae constituted the taxon comprising the largest diet component. So we conclude is that the parents take advantage of the most abundant insects locally available to feed their nestlings. The consistency of food type from location to location is evident from the uniformity of diets throughout the nestling period. The greatest difference in proportion of aquatic to terrestrial insects was seen at the Camp Henry site where the nest boxes were remote from Lake Erie compared to other sites. This again confirms the fact that tree swallow parents forage for their nestlings in the neighbourhood of their nest boxes and feed their young with the most abundant locally available group of insects.

The stable carbon isotope method can theoretically be used to determine relative proportions of terrestrial and aquatic food sources eaten by an organism. However, the analyses of birds like tree swallows, which eat different kinds of insects, must be supplemented with a method like ligature that can provide the detailed information necessary to determine which of the multitude of dietary components are dominant (Table 1). Mixing models cannot be solved if the predator's tissue signature falls outside of the range of the food signatures that have been analysed, as was the case in this study. Of the 4 food items we collected and analysed members of the Chironomidae are

especially diverse, constituting over 1200 species in North America. The stable isotope signature of different species of *Chironomus* can vary greatly, depending on their feeding habits and microhabitat. Kelly *et al.*, (2004) and Grey *et al.*, (2004) found that two species of *Chironomus*, (*C. plumosus* and *C. anthracinus*) exhibited tremendous difference in both $\delta^{13}\text{C}$ (-29.8 –56.3‰, Kelly *et al.*, 2004) and $\delta^{15}\text{N}$ (-7.8 -14.7‰, Kelly *et al.*, 2004) in two adjacent lakes. Our collections of chironomids may well have contained species other than those the swallow parents were feeding their nestlings.

Incorporation of isotopes into animals changes from individual to individual and from tissue to tissue (Martínez del Río *et al.*, 2009). Tissues have different rates of incorporating isotopes *i.e.*, some tissues such as liver and plasma proteins have higher turnover rates and so incorporate isotope signature faster compared to others such as bone and collagen. Tissues with slow turnover rates require long periods to incorporate the isotope signatures of their diet. Isotope signature incorporation rate by individuals depends on body size, growth and protein turnover. Doi *et al.*, (2007) conducted experiments with larval *Chironomus acerbiphilus* to see how changes in physiological conditions bring changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and found that the signatures changed during different growth phases of larvae. McCutchan *et al.*, 2003 reported that there were variations in $\delta^{13}\text{C}$ based on the tissue used for analysis. When muscles were analyzed for isotope fractionation value ($+1.3 \pm 0.30\text{‰}$) was higher compared to whole body fractionation ($+0.3 \pm 0.14\text{‰}$) analysis. So there will be intra and inter species differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The intra and inter species differences in incorporating isotopes from dietary inputs make the selection of tissues for analysis, especially from higher trophic level organisms, difficult.

The ligature method, which is a non-destructive technique is recommended as a very good means by which to study the diet preferences of altricial birds like tree swallows. The collars we used do not impede normal gaping behaviour or other activities like the metal collars used in some other studies. Consequently, the samples provide a true representation of the composition of insects in boluses. This method has the advantage of providing food items are intact and can be identified and easily quantified (Johnson *et al.*, 1980; Chapter 3). But the dominant size of food items in our study (mayflies, with an average length of over 30 mm) was much greater than the dominant

food size of around 10 mm. suggested by earlier studies McCarty and Winkler (1999) . We found rubber bands was easier to use than previously recommended materials like metal bands, enamelled copper wires (Johnson *et al.* 1980), pipe cleaners (Orians 1966; Walsh 1978; Johnson and Lombardo 2000) and cable ties (Mellot and Woods, 1993).

Our results also show that most parents collect the same type of food insects to feed to their juveniles, suggesting that swallows are similar to other bird species in following the optimal foraging assumption that they are energy maximizers or time minimizers. When we looked further into the caloric content of the major insect groups in the diet and caloric content in cal./g dry weight basis Ephemeroptera had the highest 5469cal./gm dry weight and Chironomidae 5424 and Formicidae (the order Hymenoptera was represented by Formicidae) 4549 but it was compared by ash free weight basis Chironomidae has the lowest of the three 5355cal./g followed by Formicidae 6247 and Ephemeroptera 6553 cal./g ash free weight (Cummins and Wuycheck, 1971).

Various other methods are devised for studying foraging methods and diet components of tree swallow nestlings by various researchers. Methods like gut content analysis where sacrificing the individuals, and methods like feces analysis and use of emetics and even digiscoping (Larson and Craig 2006) are not very effective in insectivores like tree swallows. The use of emetics also reports high mortality rate (Valera *et al.* 1997). The above methods would not give exact size of the prey items and also the amount of feeding at each time (Johnson, *et al.* 1980). Our studies suggest that ligature method is a very good method to identify the different insect prey items in the diet of altricial nestlings. Stable isotope method is very important as it give time integrated measure of food consumed by an animal. But it is very important to get the right tissue from the predator and the right prey. It is difficult to get the exact prey species in the natural habitat because animals feed on many different types of food.

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Appendix 3: Calculations for 50% (spiked mayfly) food supplement when body weight is 10 g

$$\text{Total PCB in bird (kcal b}^{-1}\text{)} = C * BW * \text{PCBinsect} * \text{ASSIM} / \text{CD insect}$$

(where C= total consumption, BW= Body weight , PCBinsect = PCB concentration in insect tissue $\mu\text{g/g}^{-1}$ and CD = caloric density of insects g^{-1} wet weight)

$$\begin{aligned}\text{For 10 g BW, the C will be} &= \mathbf{0.49 BW^{0.23}} \text{ (Nichols } et al. 2004) \\ &= \mathbf{0.49 * 10^{0.23}} \\ &= \mathbf{0.83 \text{ kcal g}^{-1} \text{d}^{-1}}\end{aligned}$$

So the total food requirement will be $0.83 \times 10 = 8.3 \text{ kcal/d}^{-1}$

$$\text{Caloric density of insects (mayflies)} = \mathbf{1.2 \text{ kcal g}^{-1}}$$

If at BW 10 g if the wrens are fed with 100% mayflies with body burden of $1 \mu\text{g/g}^{-1}$

$$\begin{aligned}\text{The body burden in the nestling will be} &= (\mathbf{8.3 * 1 * .9 (ASSIM)}) / \text{CD insect} \\ &= \mathbf{6.02 \mu\text{g/ bird}^{-1}}\end{aligned}$$

$$\text{If 50\% mayflies} = \mathbf{3.01 \mu\text{g/ bird}^{-1}}$$

$$\text{The burden per gram tissue will be } 3.01/10 = \mathbf{0.3 \mu\text{g/g}^{-1} \text{ bird tissue}}$$

$$\text{If fed with 4.2 g mayflies for 5 d, the accumulation will be} = \mathbf{0.3 \times 5 = 1.5 \mu\text{g/g}^{-1}}$$

We spiked the mayflies to produce a concentration of $20 \mu\text{g/g}^{-1}$ mayfly tissue on a dry weight basis (because their moisture was driven off after the spiking processes). The percentage of moisture in mayflies was determined as 52.5% (average) during contaminant analysis of two samples of mayflies collected in 2003 from Point Pelee National Park.

The total accumulation = contribution from food + contribution from the egg
(Table 2).

Appendix Table 2. Estimated mayfly biomass required to feed wren nestlings from age 5 d to 9 d, and the total contaminant accumulation if 50% of diet is from supplementary mayflies with a contaminant load of 20 µg/g ($\approx 0.49 \text{ BW}^{0.23}$, *total consumption /caloric density of mayflies and $\text{C}^* \text{ BW}^* \text{ PCB}_{\text{insect}}^* \text{ ASSIM/CD}_{\text{insect}}$)

Nestling age (d)	Nestling mass (g) ^a	Mass-specific daily consumption (kcal g ⁻¹ d ⁻¹)	Total daily consumption (kcal d ⁻¹)	Mass of mayflies needed (g)*	Total body burden if ½ of diet consists of supplemental, contaminated mayflies (µg/g©)
5	7	0.77	5.39	4.49	40.41
6	8	0.79	6.32	5.27	47.43
7	9	0.81	7.29	6.06	54.54
8	10	0.83	8.3	6.92	62.28
9	10.5	0.84	8.82	7.35	66.15

Total fresh biomass of mayflies required to feed one 5-day-old individual for 5 d (5d to age 9 d) is 30.09 g.

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